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Biochemistry

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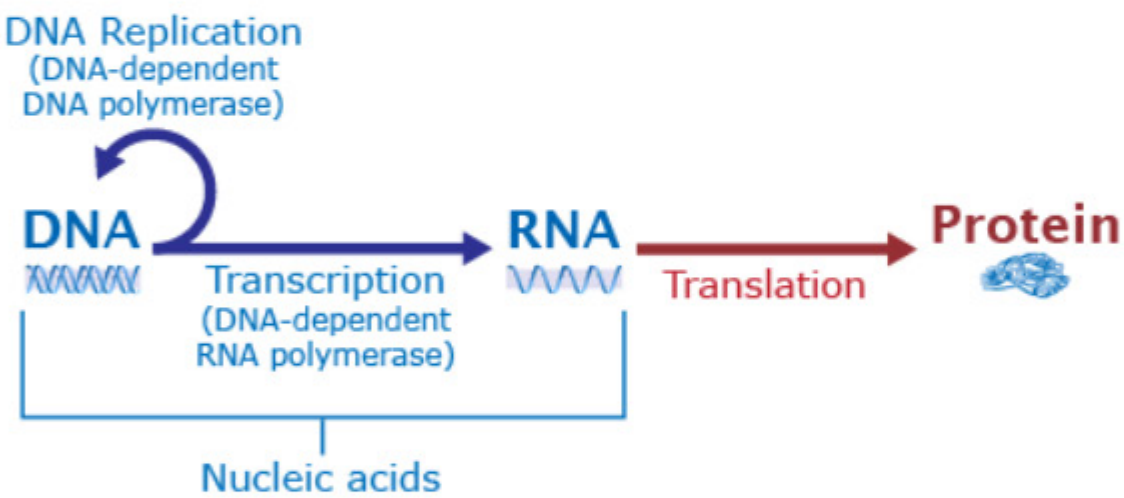
1 The Essence of Molecular Biology

In molecular biology, we connect the genotype of individuals, that is, the content of their DNA, to their phenotype, which is the expression of that genotype in their appearance and the way their bodies function. The relationship between genotype and phenotype is complex, and involves the dynamic interaction of genes, and the proteins they encode, with myriad environmental stimuli.

The genetic information on which the phenotype is built is contained in every cell in the form of DNA. *Replication* is the process by which DNA is duplicated. This process is undertaken prior to cell division to ensure that all daughter cells contain the same genetic information as the parent cell. The information in DNA is transmitted to RNA via a process called *transcription*. RNA synthesizes protein through the process of *translation,* which largely controls the appearance and function (i.e., the phenotype) of cells and organisms.



▲ **Figure 1–1.0** The Central Dogma of Molecular Biology

USMLE® Key Concepts

For Step 1, you must be able to:

- Explain the structure and characteristics of DNA and RNA.
- Describe the structural levels of DNA organization.
- Explain the chemical basis of purine-pyrimidine base pairing.
- Describe the process of nucleic acid polymerization.
- Differentiate the roles of hydrogen bonds and phosphodiester bonds in DNA and RNA structure.

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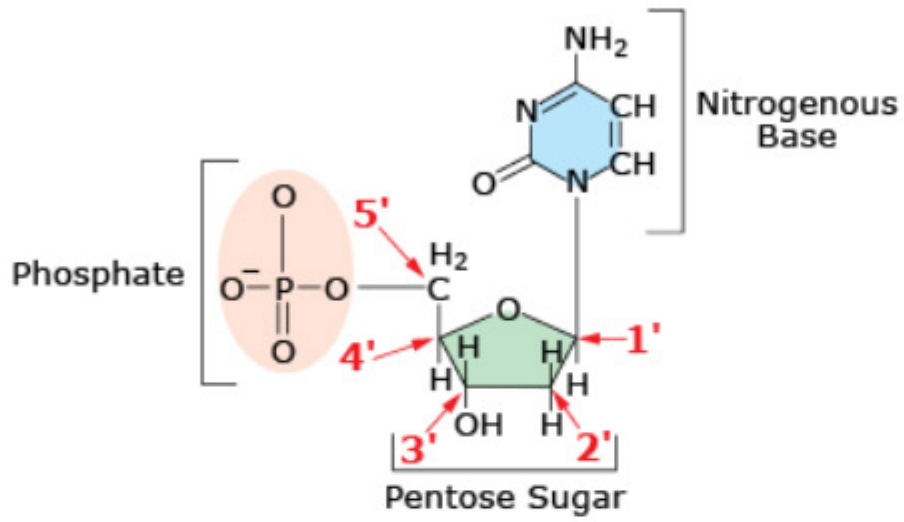
2 Nucleic Acid Structure

DNA and RNA are the two major types of nucleic acids. They are composed of nucleotides, molecules with three distinct parts:

- A five-carbon sugar, either ribose or deoxyribose. The carbons of the pentose sugar are numbered 1' through 5', clockwise, beginning with the carbon attached to the nitrogenous base.
- A nitrogenous base.
- One or more phosphate groups (attached to the 5' pentose carbon).



▲ **Figure 1–2.0** Nucleotide With Numbered Carbons on Pentose Ring

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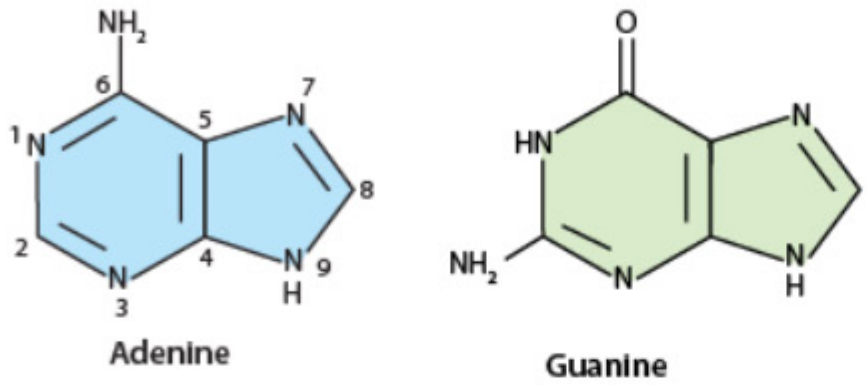
3 Nomenclature

There are two types of nitrogenous bases found in nucleic acids: purines and pyrimidines. The purines include adenine [A], guanine [G], xanthine, and hypoxanthine. Purines are composed of two rings.

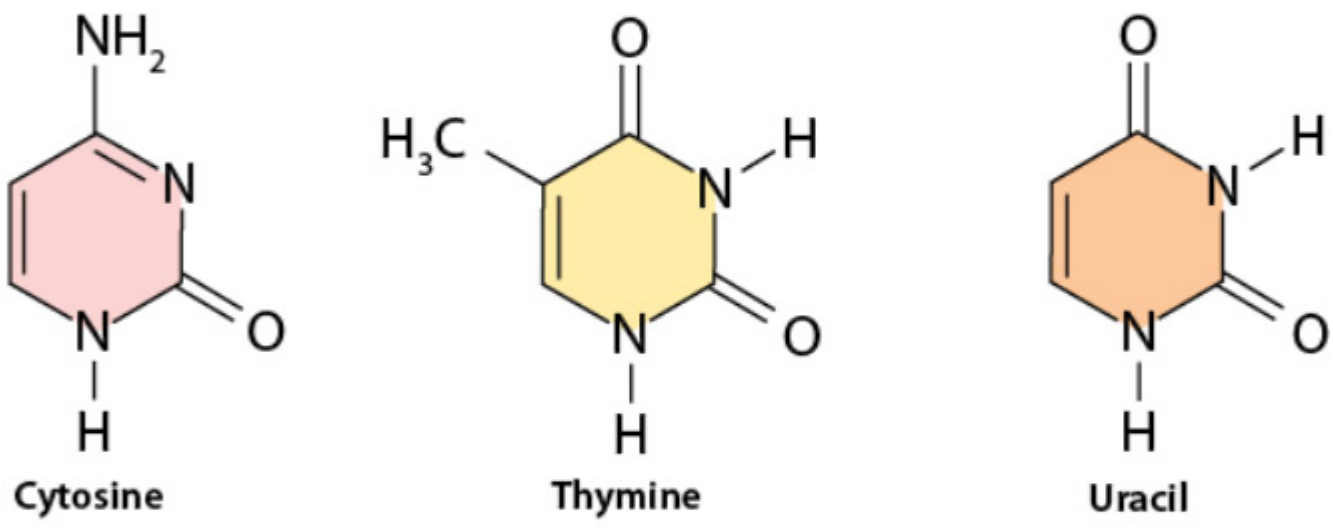


▲ **Figure 1–3.0A** Purines

Looking Ahead

Uric acid is a metabolic breakdown product of purines. In gout, high blood uric acid concentrations can lead to the precipitation of uric acid crystals in joints and tendons, leading to joint inflammation and intense pain.

The pyrimidines include cytosine [C], thymine [T], and uracil [U], and are composed of only one ring.



▲ **Figure 1–3.0B** Pyrimidines

Remember that uracil is usually found in RNA only, and thymine is usually found in DNA only.

The nitrogenous base plus a five-carbon sugar is called a nucleo*side*. Nucleosides are named according to the attached nitrogenous base. These are:

- Adenosine
- Guanosine
- Cytidine
- Thymidine
- Uridine

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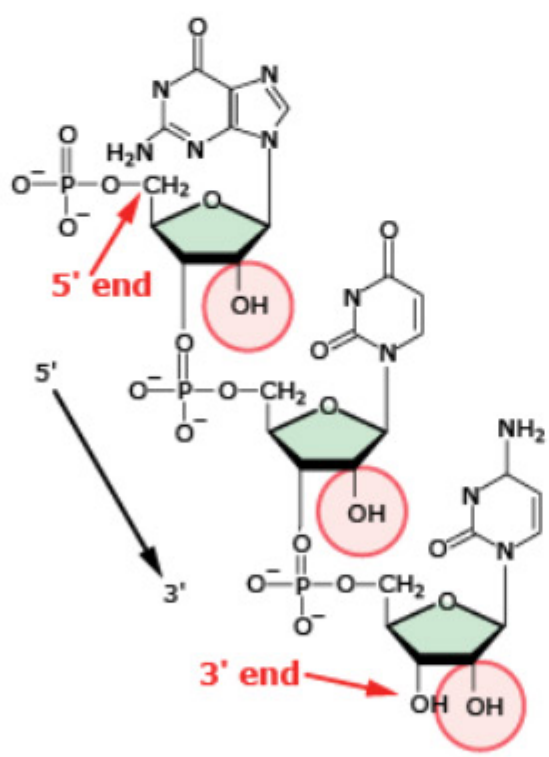
The nucleoside plus phosphate is called a nucleo*tide* and is named according to the number of attached phosphates. For example, the nucleotides formed from the nucleoside adenosine include:

- 1 phosphate = adenosine monophosphate (AMP)
- 2 phosphates = adenosine diphosphate (ADP)
- 3 phosphates = adenosine triphosphate (ATP)

▼Table 1–3.0 Nitrogenous Base Nomenclature

Bases	Nucleosides (base + sugar)	Nucleotides (base + sugar + phosphate)
Adenine	Adenosine	AMP/ADP/ATP
Cytosine	Cytidine	CMP/CDP/CTP
Guanine	Guanosine	GMP/GDP/GTP
Thymine	Thymidine	TMP/TDP/TTP
Uracil	Uridine	UMP/UDP/UTP



▲Figure 1–3.0C 2' Hydroxyl Group on RNA

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4 DNA vs. RNA

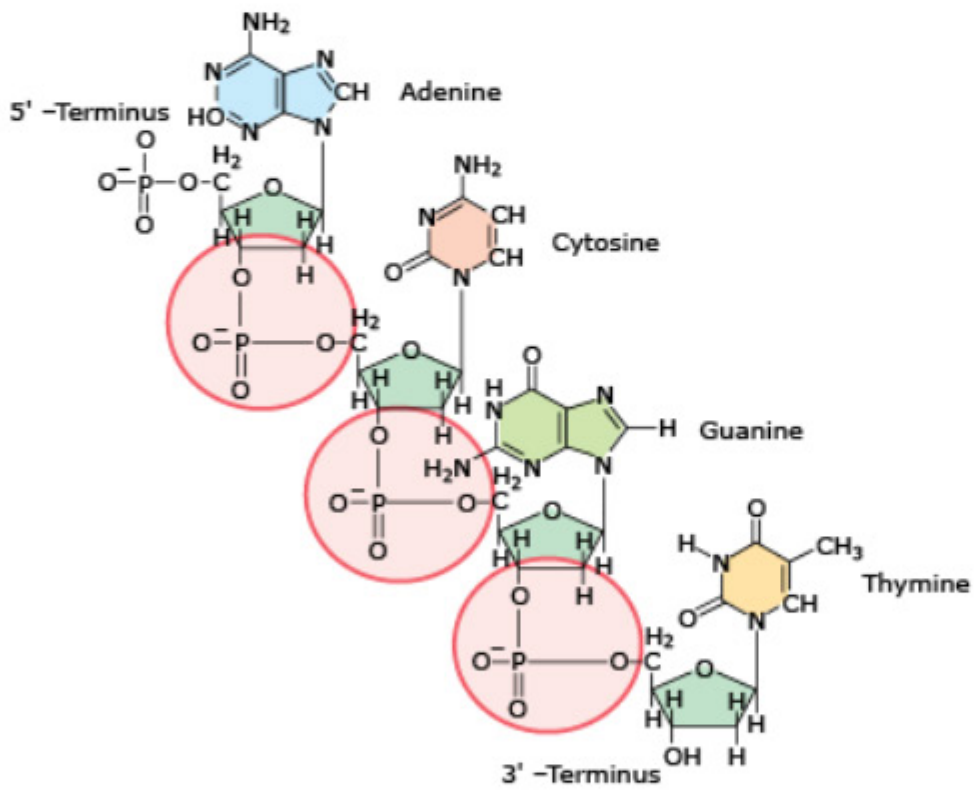
The structures of DNA and RNA differ in two major ways:

- RNA has a 2' hydroxyl (OH) group on the ribose ring, but DNA does not (hence it is named de*oxy*ribonucleic acid).
- With minor exceptions, only DNA uses thymine as a base. The corresponding base in RNA is uracil.

Both DNA and RNA use adenine, guanine, and cytosine.

5 Polymerization

Nucleic acids are polymers of nucleotides, that is, long chains of nucleotides covalently bound to one another. The connecting bond, called a phosphodiester bond, forms between the phosphate group attached to the 5' carbon of one nucleotide and the 3' hydroxyl group of another nucleotide.



▲ **Figure 1–5.0** Phosphodiester Bond

This arrangement gives nucleic acids polarity. Each polymer has a:

- Free 5' phosphate—the 5' end.
- Free 3' hydroxyl group—the 3' end.

Polymerization is the process by which one nucleotide is added to a growing nucleotide chain. This always occurs in the 5' → 3' direction, that is, the 5' phosphate of the free nucleotide is added to the 3' hydroxyl group of the growing polymer.

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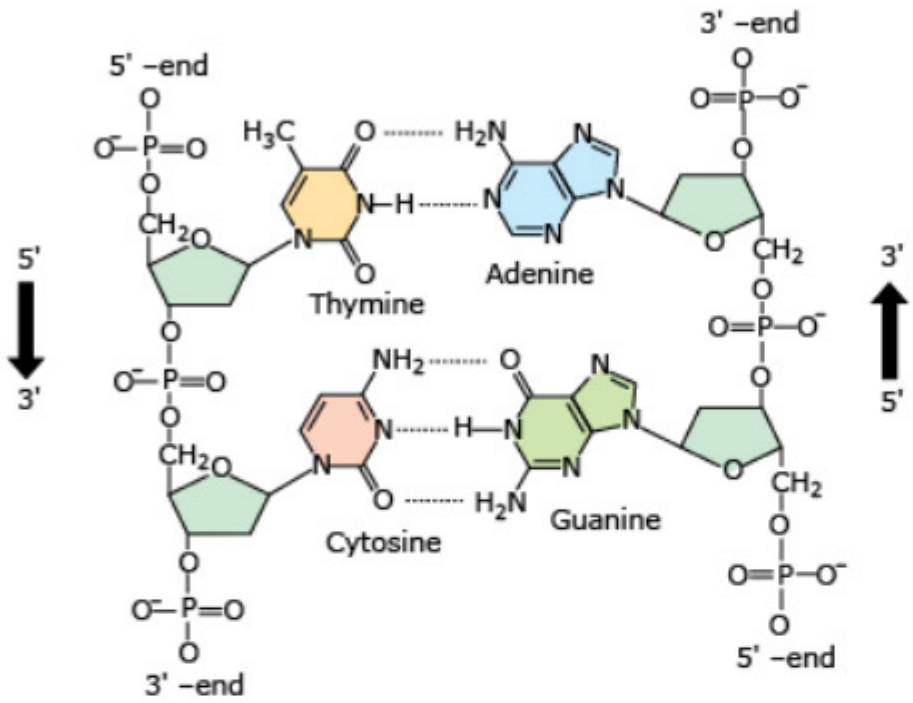
6 Base Pairing

Particular bases of DNA and RNA can form pairs with other bases through specific hydrogen bonds:

- Guanine and cytosine (G-C pair)—three hydrogen bonds.
- Adenine and thymine (A-T pair) in DNA—two hydrogen bonds.
- Adenine and uracil (A-U pair) in RNA—two hydrogen bonds.



▲ **Figure 1–6.0A** Base Pairing

The G-C pair is stronger and more stable because it has more hydrogen bonds than the A-T or A-U pairs. Because of base pairing, individual DNA strands can interact with each other, forming the characteristic double helix structure. These polymers are:

- **Complementary**: The sequence of nucleotides is constrained by the requirement of G-C and A-T interactions due to both spacing and bonding alignment. Because of this, in a double stranded DNA molecule, the total number of A nucleotides is equal to the total number of T nucleotides. Similarly, the total number of G nucleotides is equal to the total number of C nucleotides. This numerical parity is known as Chargaff's rule.
- **Antiparallel**: The complementary base pairs only form hydrogen bonds if the two chains are oriented in opposite directions.

```
5'  –AGCATTCGAAGATTC–  3'
    | | | | | | | | | | | | | | |
3'  –TCGTAAGCTTCTAAG–  5'
```

▲ **Figure 1–6.0B** Complementary and Antiparallel Structure

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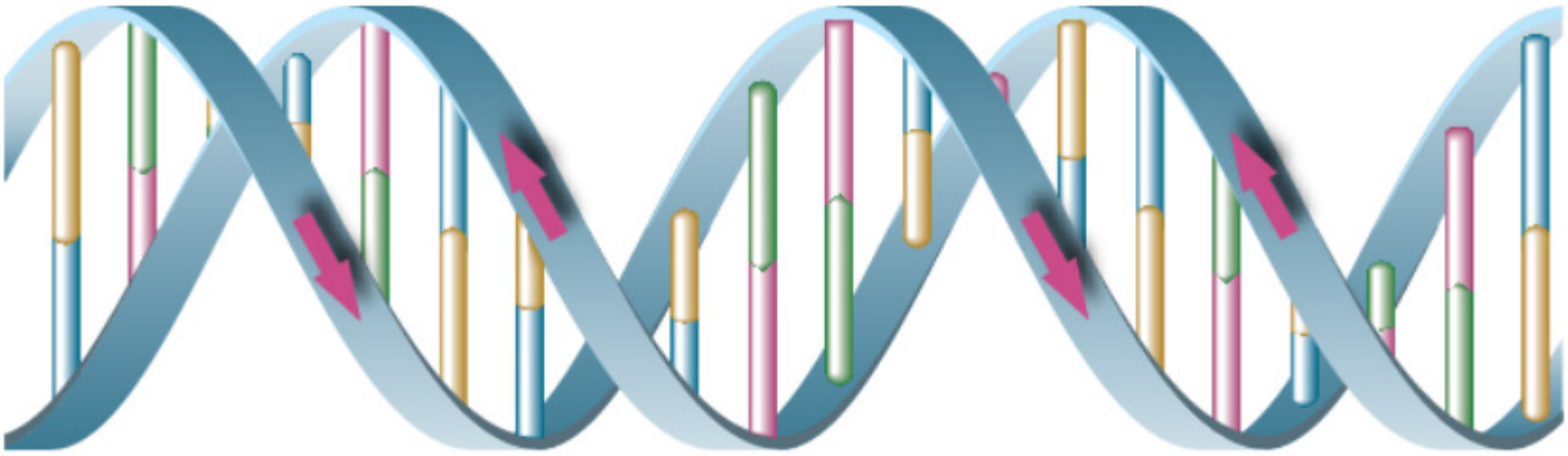
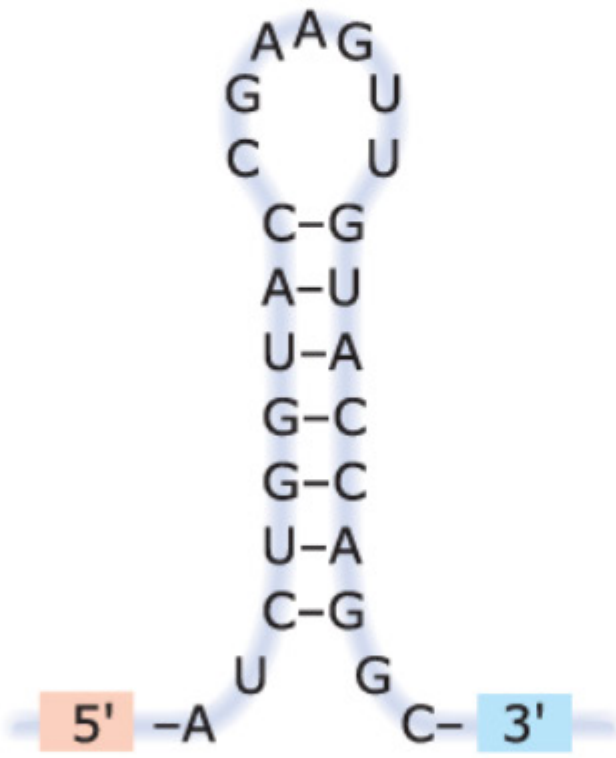


▲ **Figure 1–6.0C** DNA Double Helix

RNA is usually single-stranded (an important exception is the class of dsRNA viruses discussed in Microbiology). RNA base-pairing is typically intramolecular, that is, one part of a strand pairs with another part of the same strand. This allows individual RNA molecules to form unique three-dimensional structures such as the hairpin loop shown in Figure 1–6.0D.



▲ **Figure 1–6.0D** RNA Hairpin Loop

Important Concept

Three bonds are stronger than two. G-C bonds are stronger than A-T and A-U.

Complementary = G must pair with C, A must pair with T.

Antiparallel = Opposite direction.

Important Concept

Remember the RNA base pairs are G-C and A-U.

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7 Higher Order DNA Structure

7.1 The Size Problem

There are approximately 6×10^9 (6 billion) base pairs of DNA in the nucleus of a human cell. Stretched out, this DNA would be about 2 meters in length, yet it fits in a nucleus less than 10 µm in diameter. Therefore, the DNA has to be organized, compacted, and condensed in order to fit. The chromosomes in the cell must also be packaged in such a way that they are not damaged. The basic level of packaging is the *nucleosome*.

Important Concept

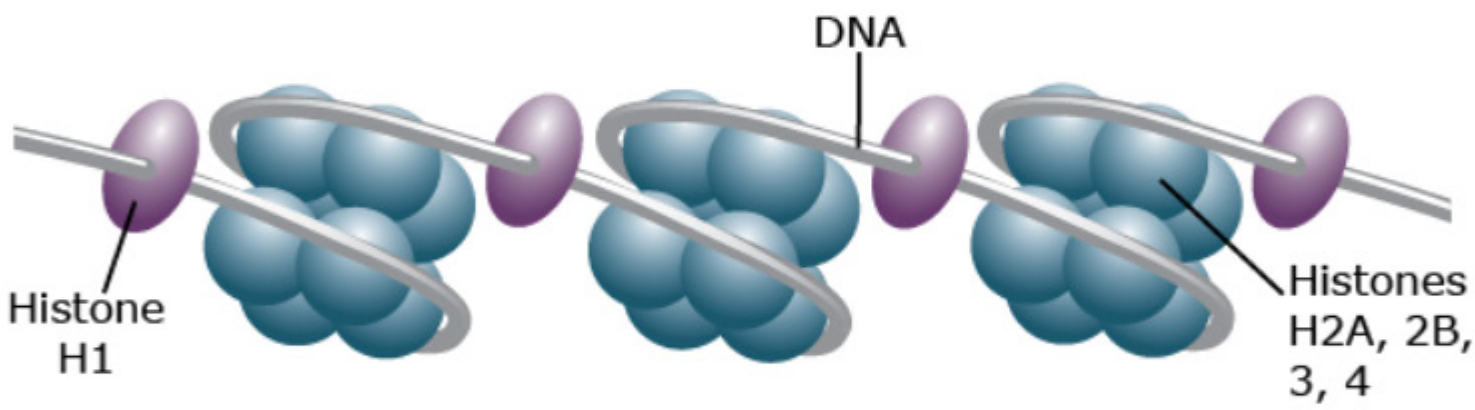
Packaging DNA

- Compacts DNA.
- Protects structural integrity.
- Is an important aspect of gene expression.

7.2 First-Order Structure: the Nucleosome

The nucleosome consists of DNA wrapped around a complex of proteins called *histones*. Histones are enriched in basic amino acids (lysine and arginine), giving them a positive charge at the pH of the cell (7.2-7.4) that allows them to bind negatively charged DNA.

The histone complex is an octomer consisting of two molecules each of histones H2A, H2B, H3, and H4. To form a nucleosome, 146 base pairs of DNA wrap around each histone complex. This structure creates a "beads on a string" appearance on electron micrographs.



▲ **Figure 1–7.2** Nucleosome

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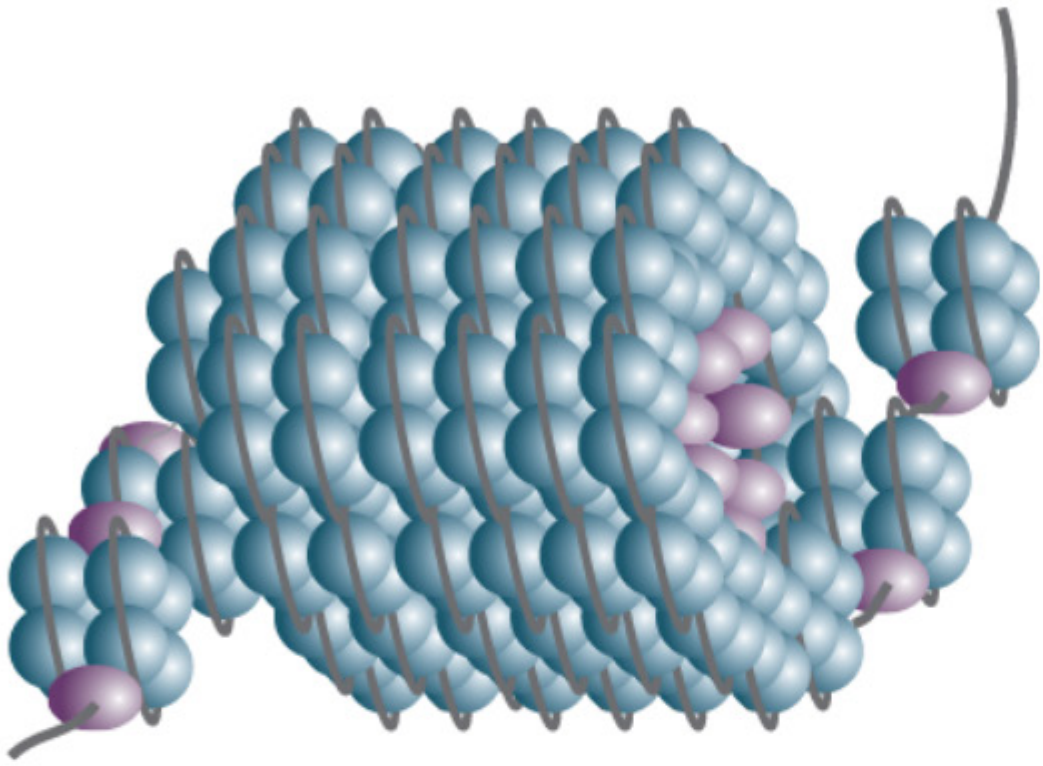
7.3 Second-Order Structure: the Polynucleosome

Individual nucleosomes are connected by a 55 base-pair spacer. This spacer is bound by another unique histone, H1. By binding to this spacer region, histone H1 helps to organize the DNA into a polynucleosome or nucleofilament.

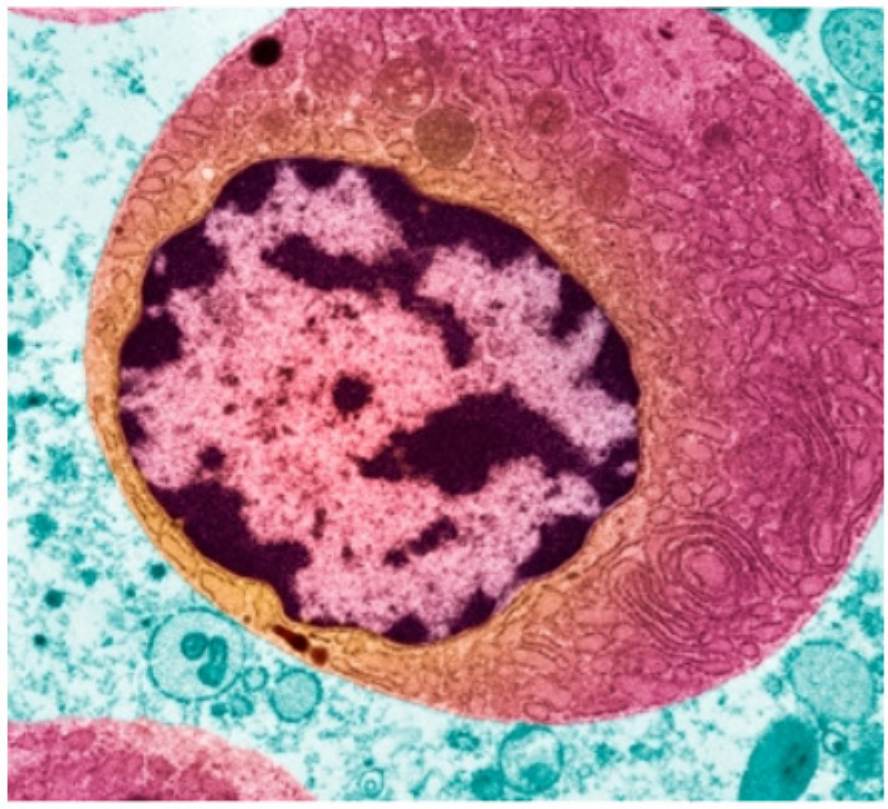


▲ **Figure 1–7.3** Polynucleosome

7.4 Higher-Order Structure

Additional condensation of polynucleosomes occurs, the result of which is *chromatin*, the DNA structure found in the nucleus. Chromatin is found in two distinct forms:

- **Heterochromatin**: More condensed and seen as denser, darker areas on electron micrographs. Typically transcriptionally inactive, meaning that these areas of DNA are not being transcribed to yield RNA.
- **Euchromatin**: Less condensed and seen as more open, lighter areas on electron micrographs. Typically more transcriptionally active, meaning that these areas of DNA are actively being transcribed to yield RNA.



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▲ **Figure 1–7.4** Chromatin

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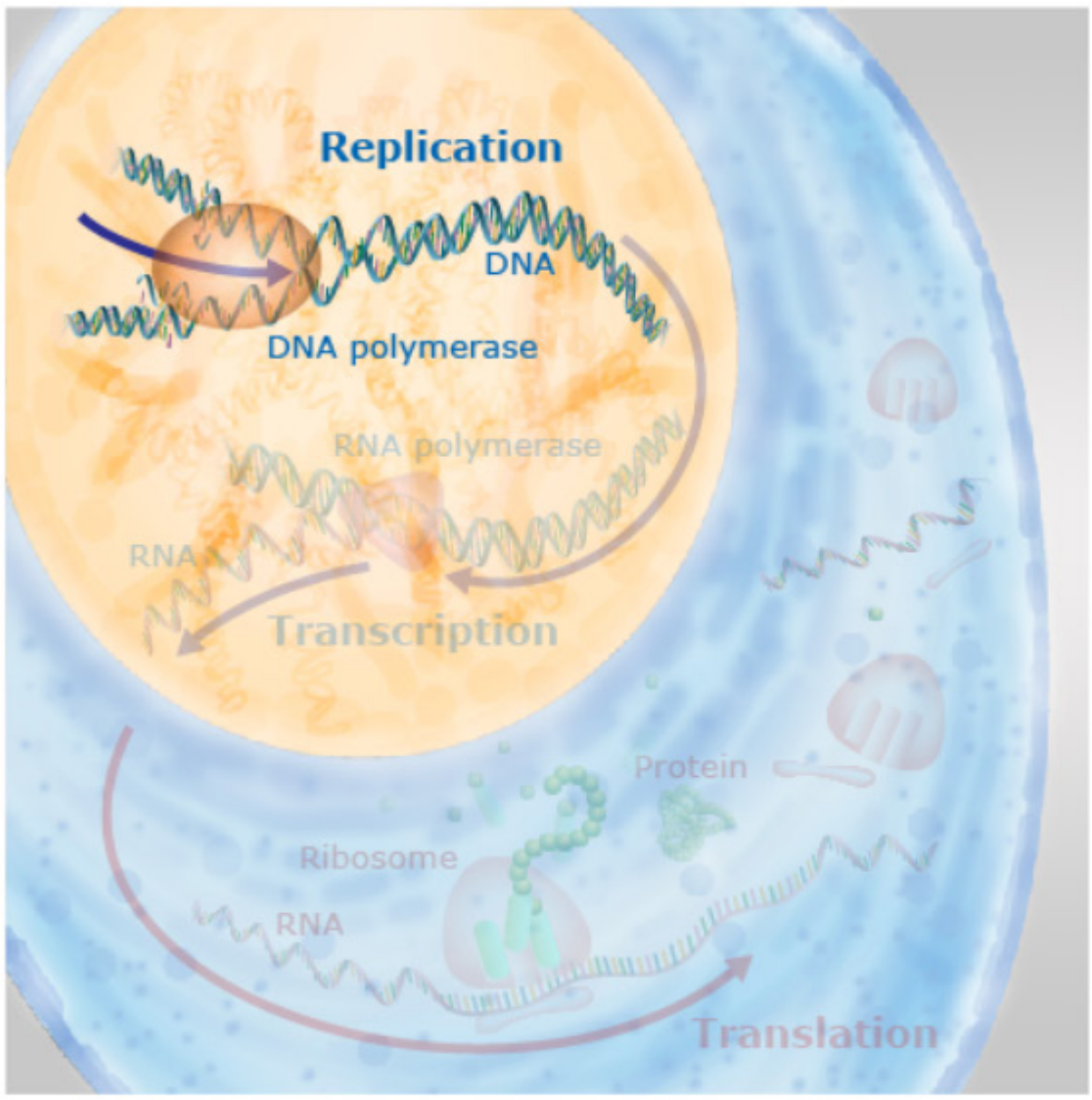
1 Overview of Replication



▲ **Figure 2–1.0** Replication

USMLE® Key Concepts

For Step 1, you must be able to:

- Describe the stages of the cell cycle and how the cycle is regulated.
- Identify the steps and major enzymes involved in DNA replication.
- Explain how DNA replication errors occur and how they are corrected.

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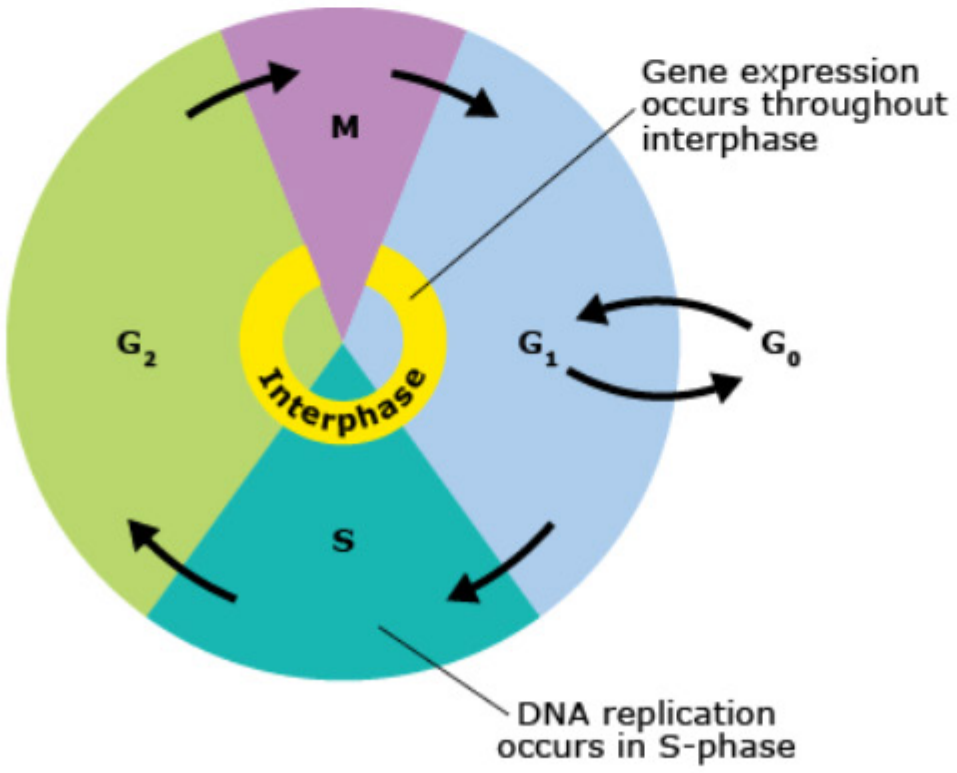
2 The Cell Cycle

The *cell cycle* is the process that a cell goes through to divide into two daughter cells. The cell cycle consists of five phases (S, G2, M, G_1, and G_0):

- S (synthesis) phase is the period of DNA replication during which the cell is making a second copy of its DNA.
- M (mitosis) phase is the period during which the cell is actually dividing. This phase is traditionally divided into five stages based on the microscopic appearance of the nuclear membrane and the chromosomes:
  - Prophase—the DNA condenses and the nuclear membrane dissolves.
  - Metaphase—the condensed chromosomes line up in the center of the cell.
  - Anaphase—the chromosomes migrate to either side of the cell, pulled by microtubules.
  - Telophase—the chromosomes decondense and a separate nuclear membrane reforms around each set of chromosomes.
  - Cytokinesis—the plasma membrane pinches off in the middle, creating two daughter cells.
- G (gap) phases:
  - G_1 is between the M and S phases.
  - G_2 is between the S and M phases. *Post-replication repair occurs during G_2.*
  - G_0 is a quiescent phase outside of the cell cycle in which no cell division takes place. Most terminally differentiated cells are in this phase.



▲ **Figure 2–2.0** The Cell Cycle

Clinical Application

Antineoplastic medications (treatments for cancer) can be cell cycle specific or nonspecific, depending on whether or not they interfere with the cell cycle.

Cell cycle specific therapies are used for fast-growing tumors.

Cell cycle non-specific therapies are used for slow-growing tumors.

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3 The DNA Replication Process

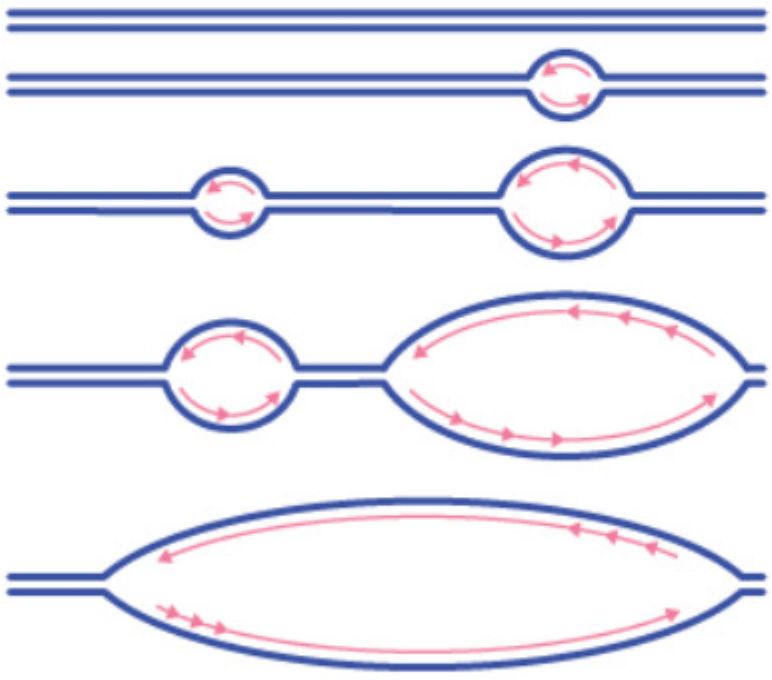
DNA replication is a five-step process:

1. Unwinding
2. RNA primer synthesis
3. DNA polymerization
4. Degrading RNA primers
5. Ligation

▲ **Figure 2–3.0** Parent Strand of DNA

3.1 Origins of Replication

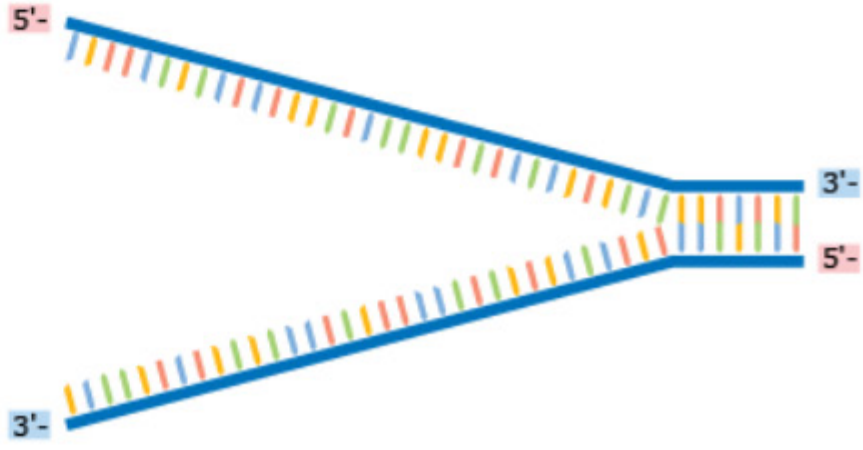
A human chromosome is tens to hundreds of millions of base pairs long. Logistically, this means that replication would be far too slow if it proceeded from only a single replication fork. In reality, replication starts at a number of different sites, termed *origins of replication*, spaced 30,000 to 300,000 base pairs apart. From each origin, a replication fork proceeds in either direction until they meet, or until they reach the end of their chromosome. The number of active origins of replication is variable. At times when rapid duplication of DNA is required (e.g., cell division of the early embryo), more origins of replication may be active.



▲ **Figure 2–3.1** Origins of Replication

3.2 Step 1: Unwinding

To be copied, the DNA helix first must be unwound and the strands separated by breaking the hydrogen bonds between the nitrogenous bases. The process is catalyzed by enzymes called *DNA helicases*.



▶ **Figure 2–3.2A** Unwound Parent DNA Strand

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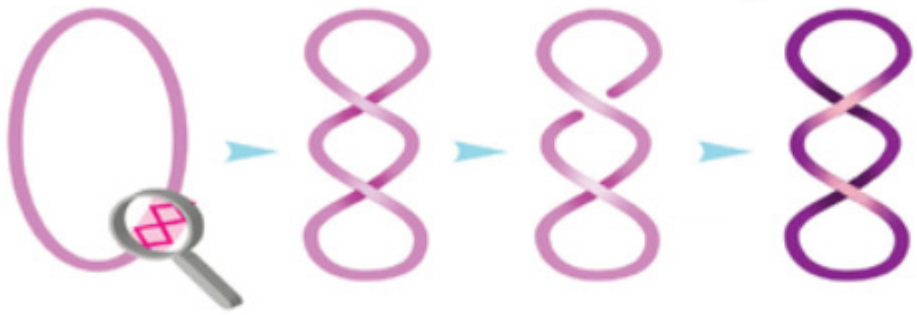
Local unwinding can cause overwinding, or supercoiling, of DNA upstream and downstream. This is prevented by proteins called *DNA topoisomerases*, which create breaks between nucleotides, allow the DNA to uncoil, and then reanneal the nucleotides.

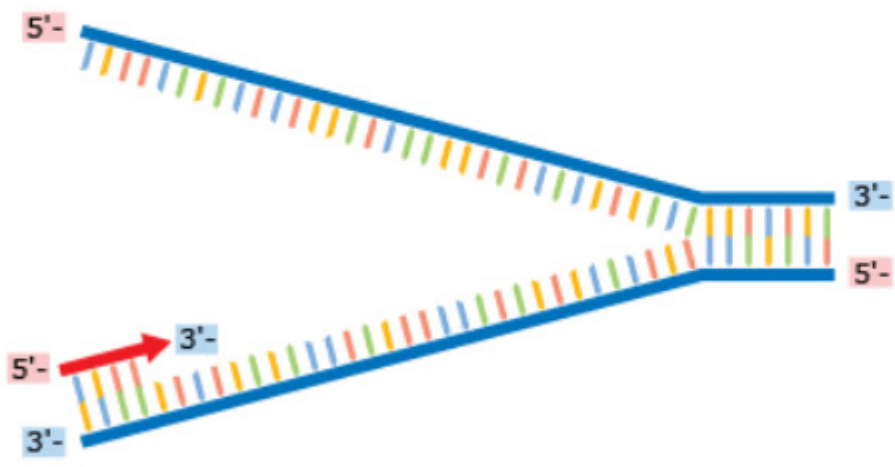
- Topoisomerase I makes single-stranded breaks.
- Topoisomerase II makes double-stranded breaks.



▲ **Figure 2–3.2B** Action of Topoisomerase

3.3 Step 2: RNA Primer Synthesis

DNA polymerases, the enzymes responsible for replication, cannot initiate synthesis of a new strand by linking free nucleotides together. In addition to a template, DNA polymerase requires a primer, a short piece of DNA or RNA with an open 3' hydroxyl, which the DNA polymerase can elongate. RNA primers are synthesized by an enzyme called RNA primase, which is a component of a DNA polymerase-α protein complex. The RNA primase synthesizes a short RNA primer (about 8–12 bp long) and the DNA polymerase-δ and polymerase-ε extend this primer approximately another 20 bases by adding deoxynucleotides. At this point, DNA polymerase-δ displaces the priming protein complex and continues to elongate the DNA.



▲ **Figure 2–3.3** RNA Primer on Parent DNA

Topoisomerase Inhibitors

Cell-cycle specific chemotherapeutics preferentially target rapidly dividing cells, such as cancer cells, by interfering with processes critical for cell division. Topoisomerases are a target of such drugs because they are needed to prevent DNA supercoiling during the process of replication. When topoisomerase activity is disrupted by drugs such as etoposide, replication stalls, leading to arrest of cell division. Ultimately, this leads to the death of cancer cells and other rapidly dividing cells. Ciprofloxacin and related derivatives inhibit bacterial topoisomerase-2, commonly referred to as DNA gyrase. These drugs are used as antibiotics.

RNA primers are required for replication but not transcription.

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3.4 Step 3: DNA Polymerization

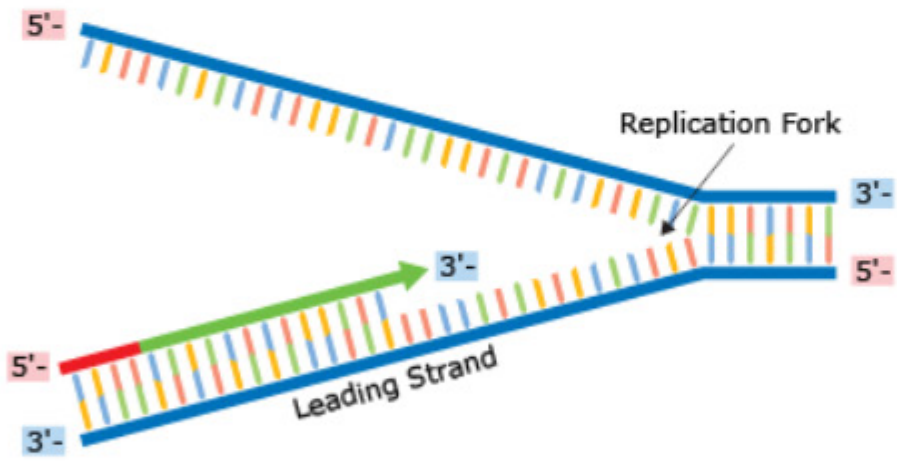
The daughter DNA strand is synthesized by creating a phosphodiester bond between the 3' hydroxyl group of the growing strand and the 5' phosphate of the next nucleotide:

- New nucleotides are in the triphosphate form dGTP, dCTP, dATP, and dTTP. Energy for the formation of the phosphodiester bond comes from breaking the high-energy phosphate bonds on the nucleotide triphosphate.

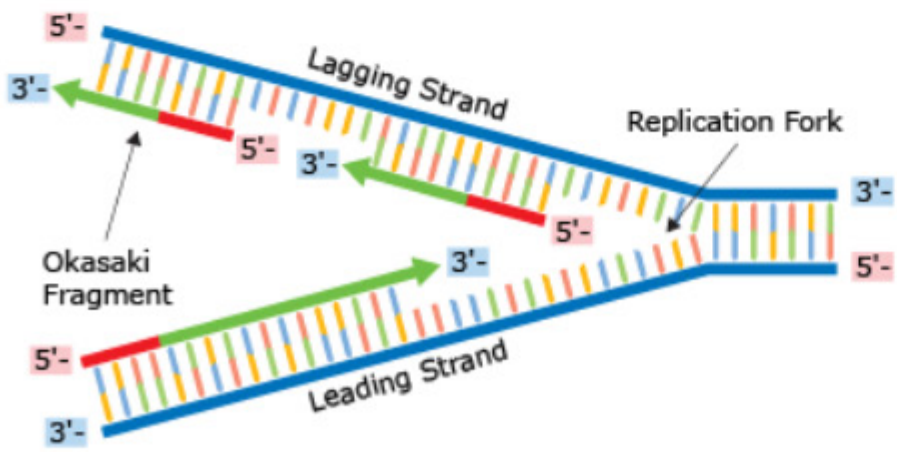
$$ATP \rightarrow AMP(DNA) + PPi \rightarrow Pi + Pi$$

- This nucleotide should be complementary (i.e., G-C or A-T) to the corresponding nucleotide in the parental strand.

Replication proceeds in the 5' → 3' direction simultaneously on both parental strands. On one strand, polymerization proceeds continuously in the direction of the replication fork, the site of DNA unwinding; this is the *leading strand*. On the other strand, called the *lagging strand,* replication also proceeds in the 5' → 3' direction, which is away from the replication fork in the lagging strand (Fig. 2–3.4A). This is done in a discontinuous fashion because the new DNA to be replicated is only exposed as the replication fork opens for replication of the leading strand. Thus, lagging strand synthesis involves the synthesis of short fragments of DNA that in humans are approximately 150 base pairs long. These are called *Okasaki fragments,* and they are later joined together through the action of DNA ligase.



▲ **Figure 2–3.4A** Synthesis of Leading Strand



▲ **Figure 2–3.4B** Synthesis of Leading and Lagging Strand Simultaneously

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3.5 DNA Polymerases

3.5.1 Prokaryotic (Bacterial) Cells

- DNA polymerase I degrades the RNA primers and fills in the resulting gap.
- DNA polymerase II participates in DNA repair.
- DNA polymerase III does the majority of synthesis and proofreading.

3.5.2 Eukaryotic Cells

▼ **Table 2-3.5** Eukaryotic DNA Polymerases

Enzyme	Function
DNA polymerase α	Priming and initial synthesis
DNA polymerase β	DNA repair
DNA polymerase γ	mtDNA replication
DNA polymerase δ	Lagging strand synthesis
DNA polymerase ε	Leading strand synthesis

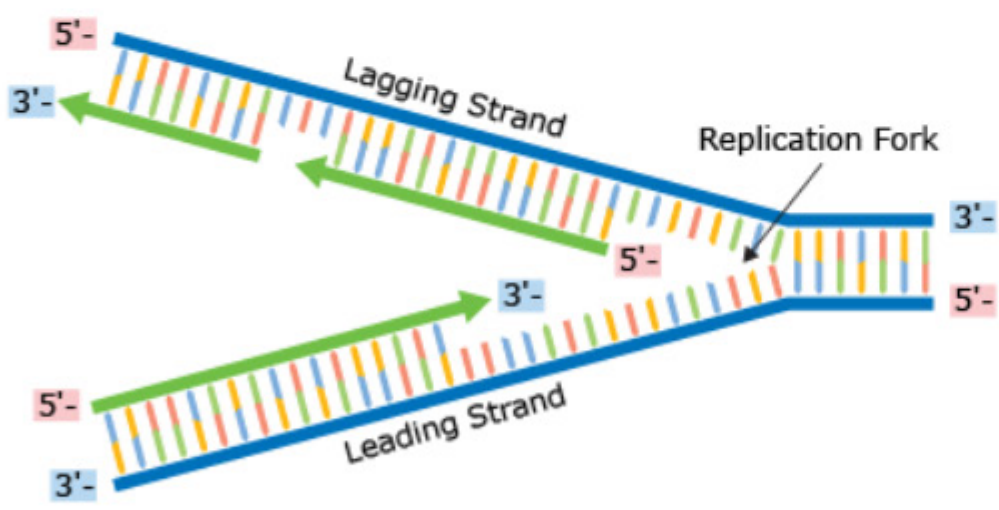
Important Concept

Prokaryotic DNA polymerases are identified by roman numerals (I, II, and III).

Eukaryotic DNA polymerases are named with Greek letters: α, β, γ, δ, and ε.

3.6 Step 4: Degrading RNA Primers

The RNA primers are removed by a 5' → 3' exonuclease, probably associated with RNase H. The resulting gap is filled in by a DNA polymerase. The identity and mechanism of this DNA polymerase still remains speculative (and is not tested on USMLE).



▲ **Figure 2–3.6** RNA Primers Degraded

Important Concept

- Nucleases break phosphodiester bonds.
- Exonucleases remove nucleotides by breaking the phosphodiester bond of the first (5' → 3') or last (3' → 5') nucleotide in a strand of DNA.
- Endonucleases break phosphodiester bonds in the middle of a DNA strand.

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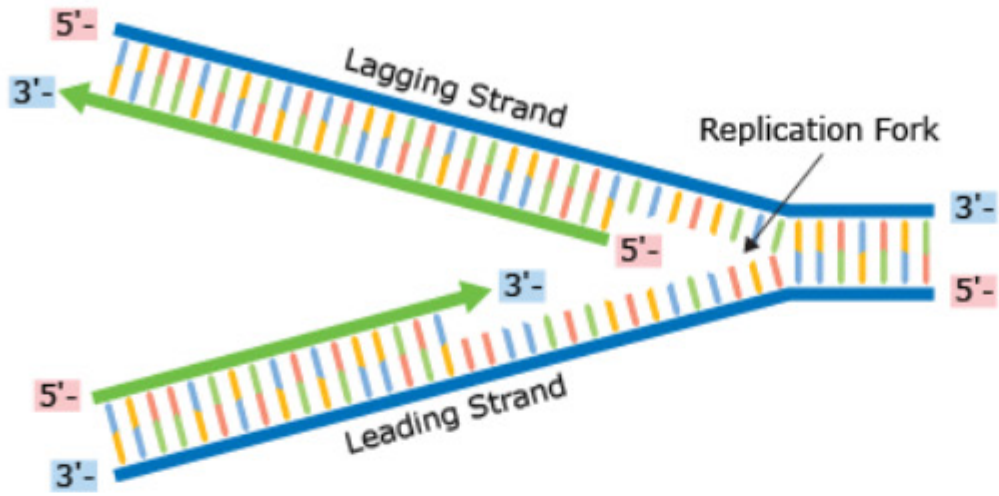
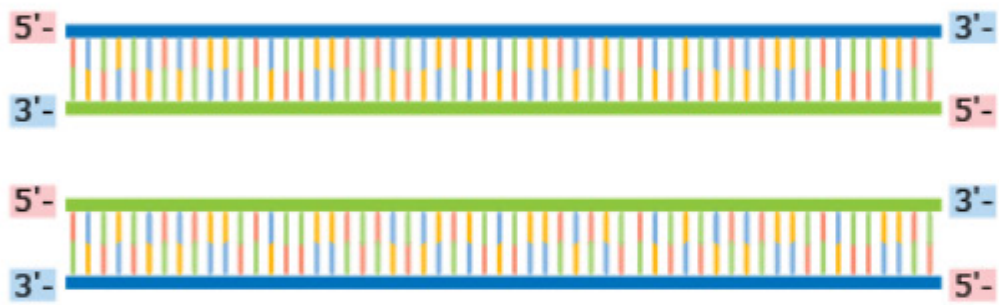
3.7 Step 5: Ligation

Ligation is the creation of phosphodiester bonds between individual DNA fragments so that the whole thing becomes one continuous strand. This is catalyzed by an enzyme called *DNA ligase*.



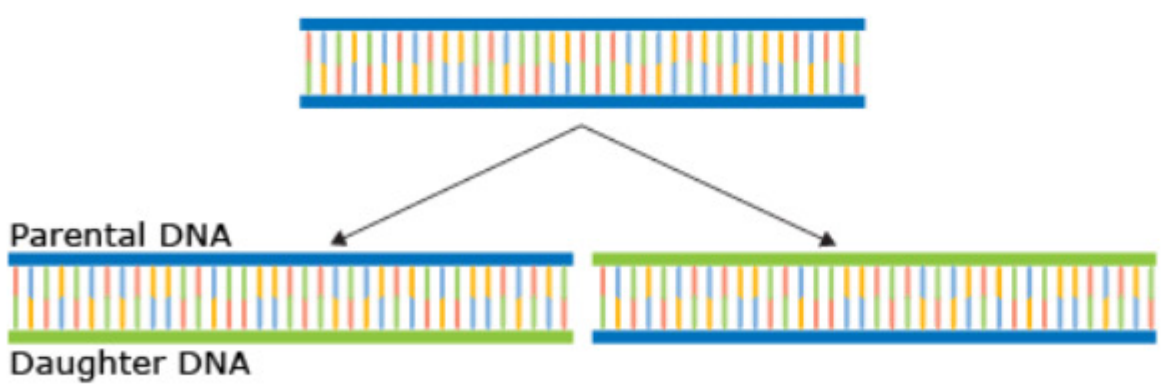
▲ **Figure 2–3.7A** DNA Ligase

This process continues until the entire strand has been replicated to form two identical daughter strands.



▲ **Figure 2–3.7B** Two Continuous Strands of DNA

DNA replication is a semi-conservative process, meaning that each daughter molecule of DNA consists of one strand of parental DNA and one strand of newly synthesized daughter DNA.



▲ **Figure 2–3.7C** Semi-Conservative Process

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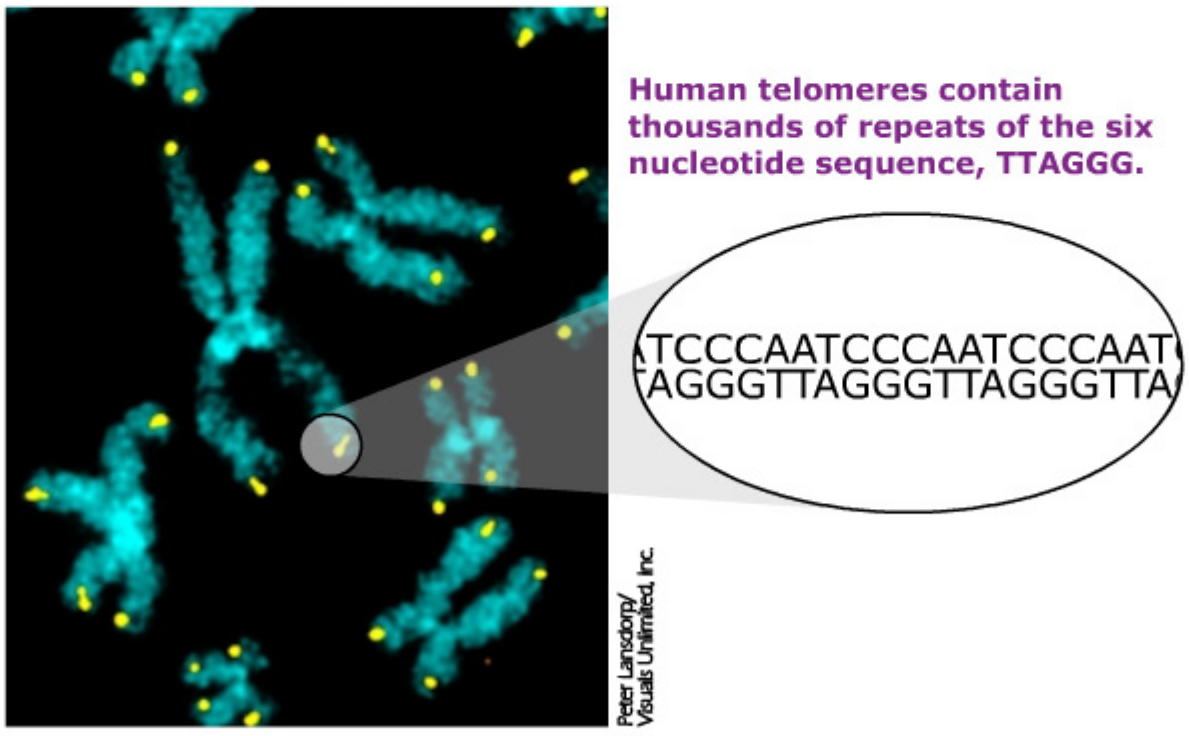
4 Telomeres

DNA polymerase cannot replicate DNA to the very end of the chromosome, meaning that the chromosome gets a little bit shorter every time a cell divides. Thus, if critical genes were at the ends of chromosomes, they would be lost during cell division. Instead, chromosome ends have *telomeres*, long stretches of repetitive sequences. In humans, this sequence is TTAGGG.

Telomeres are progressively shortened with each cell division. When the length is exhausted, the cells often become quiescent or undergo *apoptosis*—programmed cell death. Thus, the length of telomeres is one factor that determines the life span of a cell. The human genome includes a gene that encodes the enzyme telomerase which is a human reverse transcriptase. If this gene is expressed in a cell, telomerase will be able to complete the replication of the telomeres so that the chromosomes in the cell will not shorten, thus conferring on the cell a sort of immortality. This is advantageous in several circumstances:

- During embryonic and fetal life, when very high rates of cell division are required to form a healthy newborn from a single fertilized ovum.
- Throughout life in stem cells that may also have a high rate of cell division, such as the pluripotent stem cells that replace red and white blood cells.

In many types of cancer cells, the gene for telomerase has been re-activated inappropriately.



▲ **Figure 2–4.0** Telomeres

Clinical Application

New medications called telomerase inhibitors are being created—they aim to stop cancer cell proliferation by inhibiting telomerase.

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5 DNA Editing

The process of replication is not perfect. DNA polymerase makes mistakes, usually by mismatching nucleotide bases. The error rate is ~1 per 10^5 (100,000) base pairs. This means in each replication of the human genome (6×10^9 base pairs), there are 60,000 errors, a rate that would prevent an organism from functioning.

Most of these errors are corrected during replication. The DNA polymerase complex contains a 3' → 5' editing exonuclease. This enzyme detects nucleotide mismatches as they occur, and removes the mismatched nucleotide by breaking its phosphodiester bond to the growing DNA strand. DNA polymerase is then able to replace this nucleotide with one that matches the parental strand correctly. This proofreading activity reduces the error rate by 100 times, to about 1 in 10^7 base pairs.

▲ **Figure 2–5.0A** DNA Editing

Additional proofreading occurs after replication. The final error rate is 1 in 10^9 nucleotides, or about 6 errors per human genome. This error rate is not only tolerable, but adaptive, as it provides much of the variation between individuals that is important for adaptation and survival of the species.

Important Concept

Correction of DNA mismatching starts with exonuclease and is followed with polymerase.

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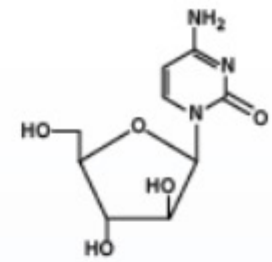
Clinical Application

Nucleotide Analogs

Because replication is critical for cell division, blocking DNA replication can be used as a treatment for diseases that require active cell division, such as cancer and viral infections. One way to do this is to use nucleotides that are modified in ways that interrupt the normal function of DNA replication.

Cytosine Arabinoside (Cytarabine)

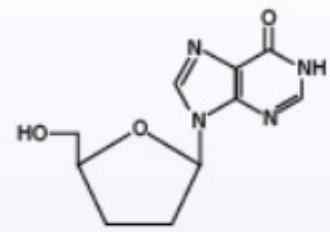
The deoxyribose sugar of cytidine is replaced by another sugar, arabinose. This modified nucleotide inhibits DNA synthesis. This is used as a chemotherapeutic agent for cancer.



▲ **Figure 2–5.0B**
Cytosine Arabinoside

2',3'-Dideoxyinosine (DDI, Didanosine)

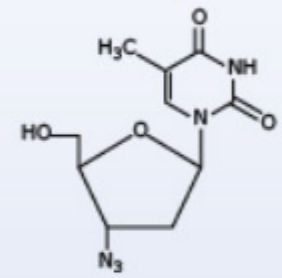
This is a nucleotide modified to remove the 3' hydroxyl group. When incorporated into a DNA strand, this halts replication because a new phosphodiester bond cannot be created without the 3' hydroxyl group. This is used to treat HIV infection.



▲ **Figure 2–5.0C**
2',3'-Dideoxyinosine

Zidovudine (AZT)

This is a nucleotide that is modified by exchanging the 3' hydroxyl group for an azide (N_3) group. Similar to DDI, this prevents the formation of phosphodiester bonds and halts replication. This is also used to treat HIV.



▲ **Figure 2–5.0D**
Zidovudine

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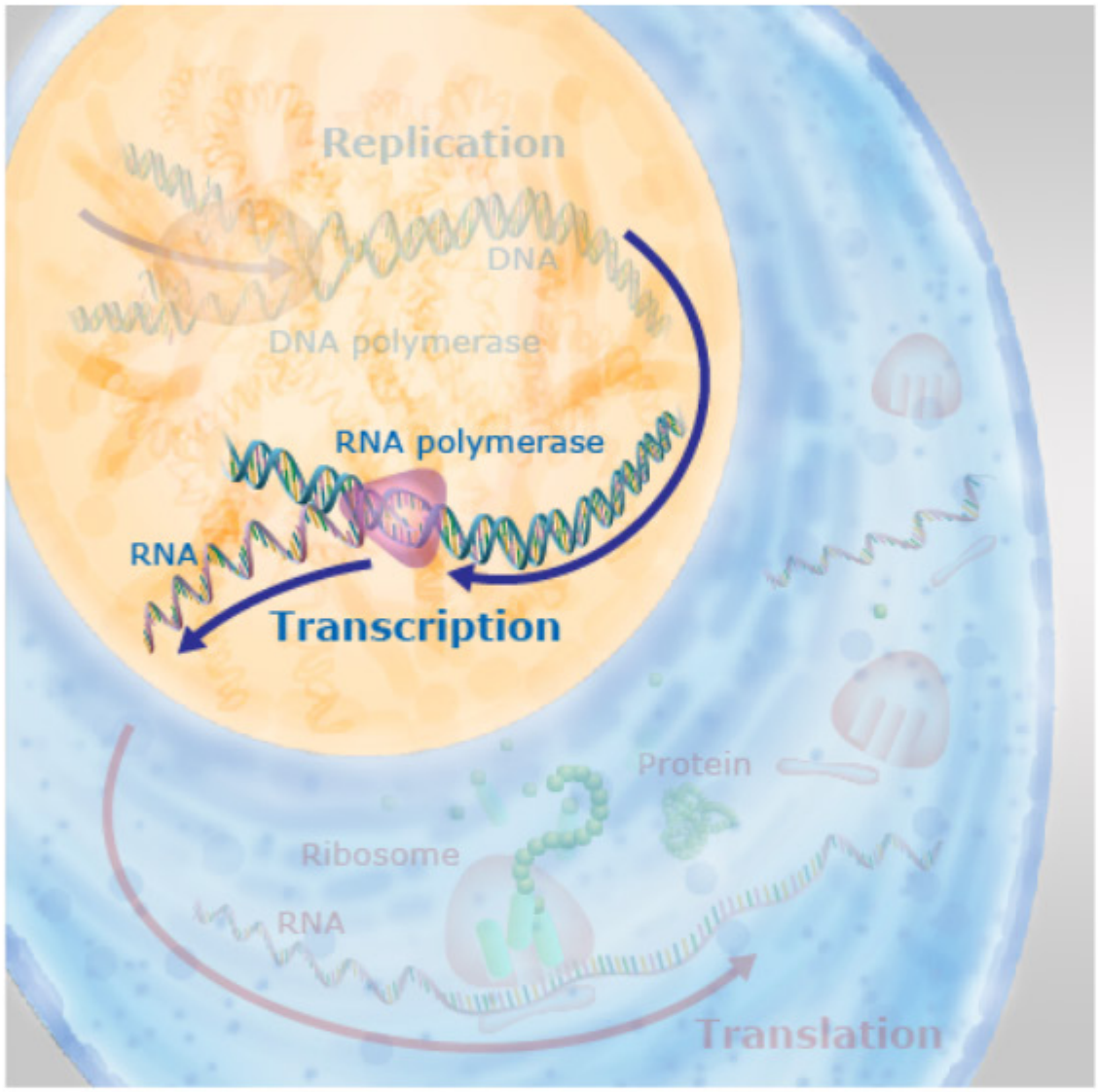
1 Overview of Transcription



▲ **Figure 3–1.0** Transcription

Transcription is the process by which an RNA copy is made from a DNA template. Conceptually, the process is similar to making multiple copies of a page (the RNAs) from a book (the DNA gene), using a copying machine (an RNA polymerase).

Transcription is catalyzed by *RNA polymerase,* requiring:

- A template, usually DNA. There are some exceptions in RNA viruses, which can use RNA-dependent RNA polymerases.
- Ribonucleotide triphosphates.

Similar to DNA replication, energy is provided by the breaking of high-energy phosphate bonds.

USMLE® Key Concepts

For Step 1, you must be able to:

- Describe in detail the structure of genes and the role of gene structures in regulating transcription.
- Explain how DNA is transcribed to RNA.
- Describe the purpose and mechanisms of RNA processing.
- Identify the cellular mechanisms that control gene expression through transcription.

Clinical Application

Rifampin is a medication used to treat tuberculosis and *Neisseria meningitidis* infections. Rifampin works by inhibiting DNA-dependent RNA polymerase.

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2 Types of RNA

There are four different types of RNA that can be created by transcription:

- **Heterogeneous nuclear RNA (hnRNA) and messenger RNA (mRNA):** Genes that encode proteins are all transcribed by RNA polymerase II. The RNA primary transcript is modified in the nucleus before it is released into the cytoplasm to be translated. During processing in the nucleus, most of the primary transcripts become shorter. During this time, one can find RNAs of different sizes in the nucleus. Collectively, these are referred to as heterogeneous nuclear RNA (hnRNA). By the time they are fully processed and released in to the cytoplasm, they will be referred to as mature messenger RNA (mRNA).
- **Ribosomal RNA (rRNA):** This RNA is incorporated into and functions within ribosomes, which are important structures in protein synthesis (translation). In eukaryotic cells, most of the rRNA genes are transcribed in the nucleolus by RNA polymerase I. An important exception is the 5S rRNA, whose gene is transcribed by RNA polymerase III in the nucleoplasm.
- **Small nuclear RNA (snRNA):** This RNA is produced by RNA polymerase II and combines with protein components to form snRNP (small nuclear ribonucleoprotein). These snRNPs, also known as *spliceosomes*, catalyze the removal of introns from the primary transcript.
- **Transfer RNA (tRNA):** tRNA is responsible for decoding RNA during translation to form proteins. Transfer RNA is produced by RNA polymerase III.

Important Concept

hnRNA and mRNA: RNA polymerase II

rRNA: RNA polymerases I and III

snRNA: RNA polymerase II

tRNA: RNA polymerase III

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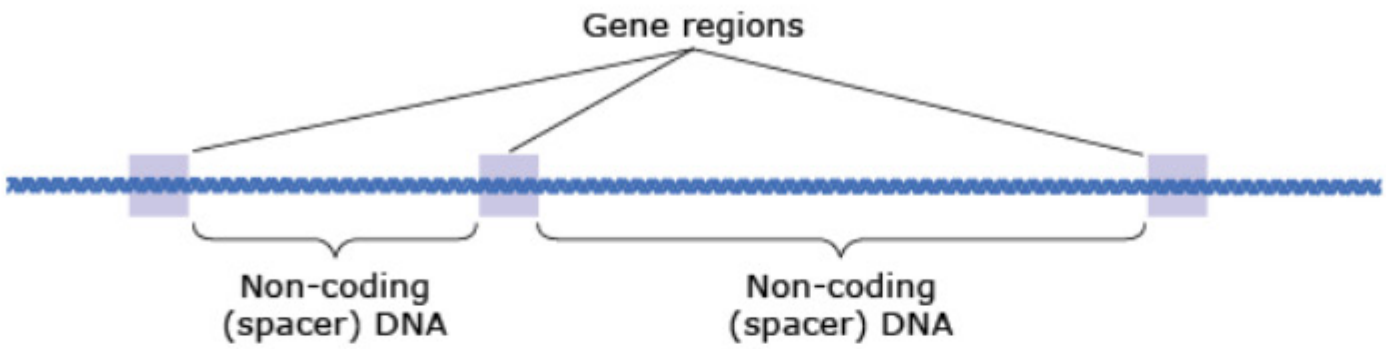
3 Genes and Non-coding Regions of DNA

Transcription is the first step in gene expression. The process is catalyzed by a DNA-dependent RNA polymerase that must first distinguish a gene region from the large amount of non-coding DNA that makes up a major part of chromosomes.



▲ **Figure 3–3.0** Genes and Non-coding Regions

It is estimated that non-coding DNA accounts for approximately 98% of the chromosomal DNA. Gene regions therefore must have molecular tags that can be recognized by RNA polymerases and by transcription factors that assist in producing an active transcription complex. RNA polymerases bind to a promoter region associated with the transcription unit.

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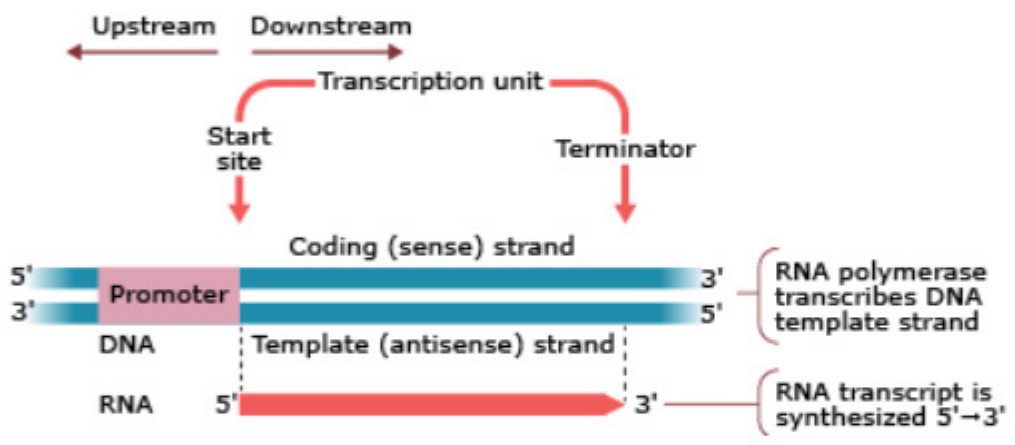
4 Gene Structure

In order to understand transcription, we have to be familiar with the structure of a gene. A gene consists of:

- Transcription unit
- Promoter
- Enhancers and silencers
- Terminator

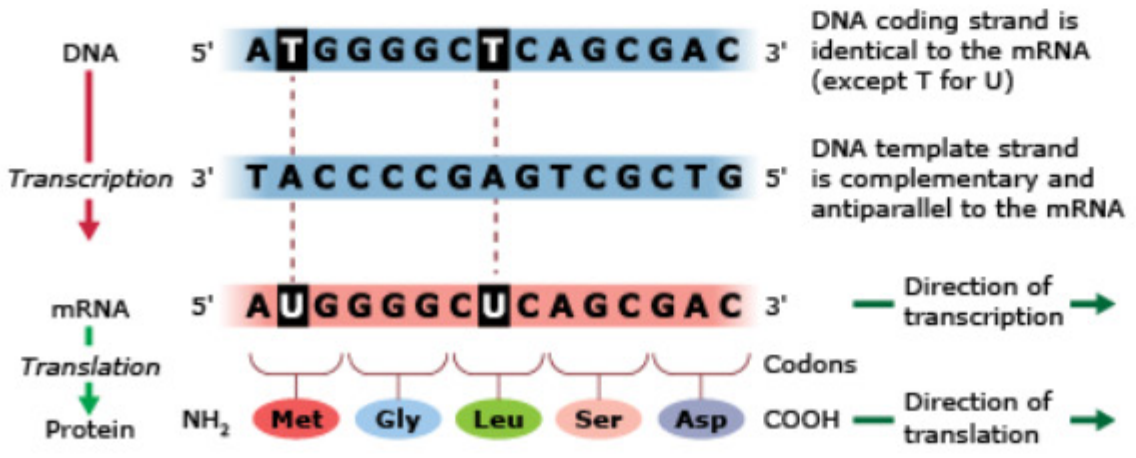
4.1 Transcription Unit

The transcription unit is the sequence of the gene region that is transcribed to produce the RNA. In transcription, unlike DNA replication, only one of the two strands of DNA in each gene region is used as the *template or antisense strand*. The other strand, not used in transcription, is paradoxically named the *coding or sense strand*. Figure 3-4.1A shows these elements and their relationship in transcription.



▲ **Figure 3–4.1A** Transcription Unit

Figure 3-4.1B illustrates why the strand *not used* in transcription is referred to as the coding strand. A brief comparison of the nucleotide sequence of the coding strand and the newly transcribed mRNA will show that the coding strand of the DNA and mRNA are identical (except where mRNA's uracil replace DNA's thymine). Both display the codons for the amino acid sequence of the protein encoded by this gene. Because of this, gene sequences are conventionally from the coding strand and written 5' to 3'.



▲ **Figure 3–4.1B** Comparison of Coding Strand and Transcribed mRNA

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The transcription unit begins with the first nucleotide introduced into the RNA, by convention referred to as the +1 base. All other nucleotides in the gene are then referenced sequentially from that site. If a nucleotide lies before (5' or upstream) the +1 base, it is given a negative number and it is not transcribed into the new RNA. Similarly, if the nucleotide has a positive number, you should recognize that it is within the transcription unit and will be found in the RNA transcript.

4.2 Exons and Introns

A major characteristic of almost all eukaryotic transcription units is the presence of *exons (expressed sequences)*, which contain codons for the amino acids found in the final protein product, and *introns (intervening sequences)* that, although they are transcribed, do not code for any amino acids in the final protein product.

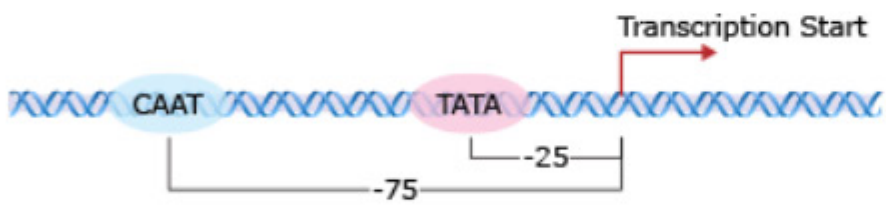
The exons are ordered in the same sequence as are the protein domains for which they code in the final protein product.

4.3 Promoter

Because the vast majority of chromosomal DNA is non-coding, gene regions must have *promoters,* molecular tags that can be recognized by RNA polymerases and the transcription factors that assist in producing an active transcription unit.

RNA polymerase, along with transcription factors, bind to the promoter. The promoter is approximately 100 bp long with two important sequences:

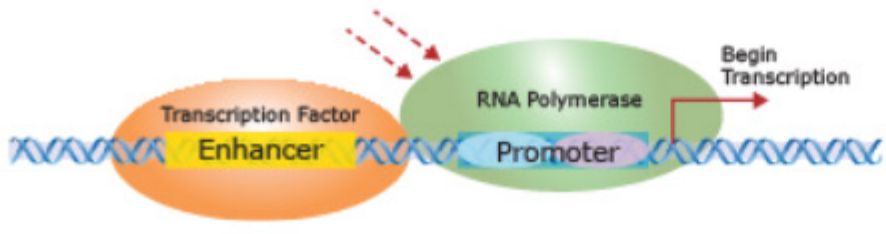
- TATA box: ~25 bp before (5' or upstream) the transcription start site
- CAAT box: ~75 bp before (5' or upstream) the transcription start site



▲ **Figure 3–4.3** Promoters

4.4 Enhancers and Silencers

Enhancers are DNA sequences that bind specific proteins called transcription factors. These sequences are typically upstream of the transcription unit, but can be just about anywhere, including within introns. When an enhancer binds with a transcription factor, the presence of the complex increases the possibility that RNA polymerase will bind to the promoter and begin transcription of that gene.



▲ **Figure 3–4.4A** Enhancer

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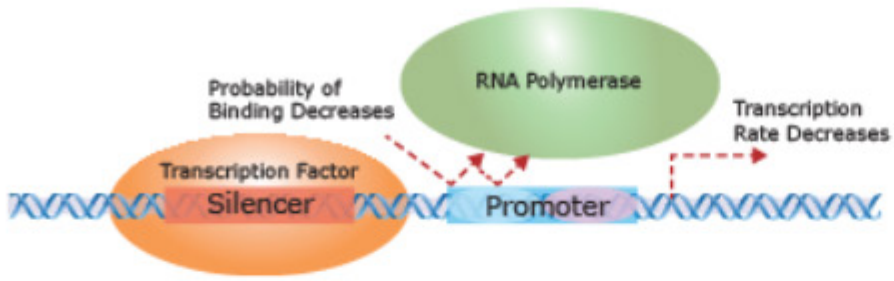
A silencer has the opposite effect on transcription. When a silencer sequence binds with a transcription factor, the probability of RNA polymerase binding with a promoter decreases and thus the rate of transcription of that gene decreases. A major mechanism by which cells respond to external inputs is by changing the levels of transcription factors present in particular cells at particular times.



▲ **Figure 3–4.4B** Silencer

4.5 Terminator

Transcription stops at a *terminator sequence*. In bacteria, the terminator sequence causes the RNA to form a hairpin stem-and-loop structure that terminates transcription.

The terminator sequence for transcription in eukaryotic cells is not yet completely understood. There appear to be several different strategies for terminating transcription.

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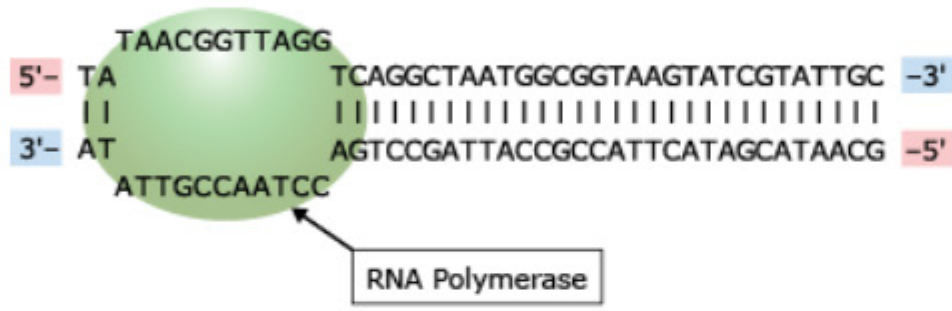
5 Transcription Process

Transcription is a four-step process:

- Binding
- Initiation
- Elongation
- Termination

5.1 Binding

RNA polymerase binds to the double-stranded promoter and unwinds a short stretch of DNA to create a small bubble. This transcription bubble is an area in which the hydrogen bonds between bases on opposite strands are broken. The bubble allows RNA polymerase access to the template DNA.

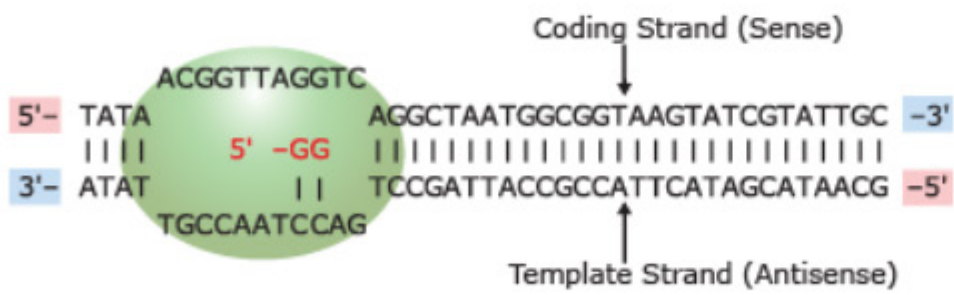


▲ **Figure 3–5.1** Binding

5.2 Initiation

Unlike DNA polymerase, RNA polymerase does not require an existing 3' hydroxyl group. RNA synthesis starts with a purine—either adenosine (A) or guanosine (G)—which means that the transcription start site on DNA is either a thymidine (T) or cytidine (C).

The next nucleotide of the sequence then hydrogen bonds with the corresponding nucleotide in the DNA template. Initiation is completed when a phosphodiester bond is created between these first two nucleotides.



▲ **Figure 3–5.2** Initiation

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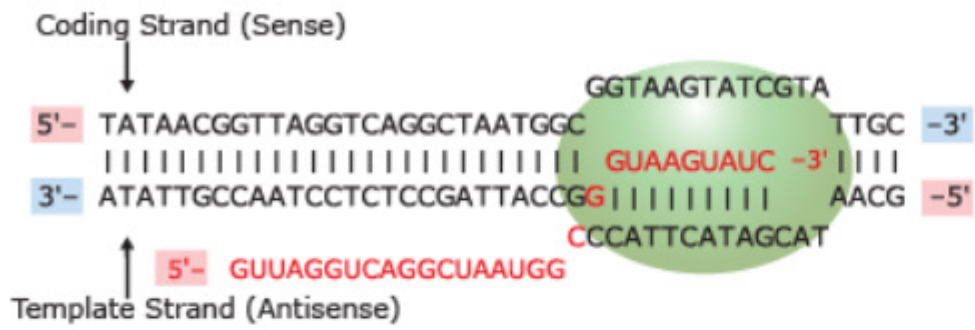
5.3 Elongation

RNA polymerase elongates the growing RNA strand in the 5' → 3' direction, forming an antiparallel and complementary copy of the DNA template. As the polymerase proceeds to elongate the RNA transcript in the 5' → 3' direction, the DNA duplex re-forms behind it and the growing RNA is released as a single strand. This step can be blocked by drugs or toxins, such as actinomycin D or α-amanitin, which can kill cells by blocking transcription.



▲ **Figure 3–5.3** Elongation

5.4 Termination

When the RNA polymerase reaches the appropriate termination sequence, it releases from the DNA template and the DNA duplex re-forms. The resulting RNA molecule is hnRNA, which must then be processed to form mRNA.

▲ **Figure 3–5.4** Termination

Actinomycin D is an antineoplastic drug used to treat:

- Wilms tumor
- Rhabdomyosarcoma
- Ewing sarcoma
- Gestational trophoblastic neoplasia

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6 RNA Processing

Before they can be translated, the hnRNA primary transcripts must be processed to form mRNA. There are three processing steps:

- 5' capping
- 3' polyadenylation
- Intron removal and splicing

6.1 5' Capping

Heterogeneous nuclear RNA is capped at the 5' end with a modified 7-methylguanosine nucleotide. The linkage between these nucleotides is not a typical phosphodiester bond; rather, the 5' phosphate of the first hnRNA nucleotide is bonded to the 5' diphosphate of 7-methylguanosine. This cap has two functions:

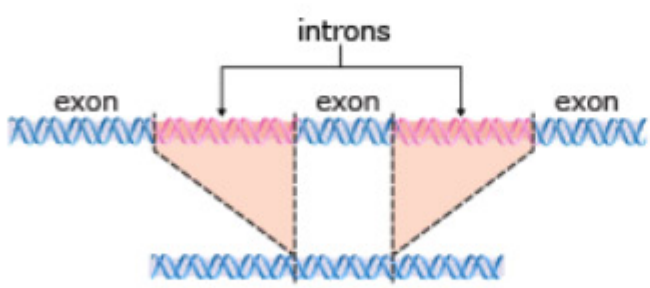
- The 5' cap protects the RNA from nucleases.
- The cap also plays a role in correct ribosome assembly when translation of the mRNA begins in the cytoplasm.

6.2 3' Polyadenylation

The 3' end of the hnRNA is trimmed by an endonuclease. A poly-A polymerase then adds to a consensus polyadenylation site in the 3' untranslated region of the RNA. At this point, a long stretch of untemplated adenylate residues is added to the end of the RNA. This functions to protect the RNA from degradation by 3' → 5' exonucleases, further extending the half-life of the RNA.

6.3 Intron Removal and Splicing

The introns need to be removed from the hnRNA so that the protein-coding exons are placed together in the right sequence for proper protein coding. This splicing is catalyzed by a complex of proteins and small nuclear RNAs (snRNAs) called the spliceosome (alternatively, referencing its composition, a small nuclear ribonucleoprotein or "snurp").



▲ **Figure 3–6.3A** Introns Removed From mRNA

The spliceosome recognizes the starting and ending sequences of an intron, which are always GU and AG, respectively.

Important Concept

Spliceosomes recognize GU and AG.

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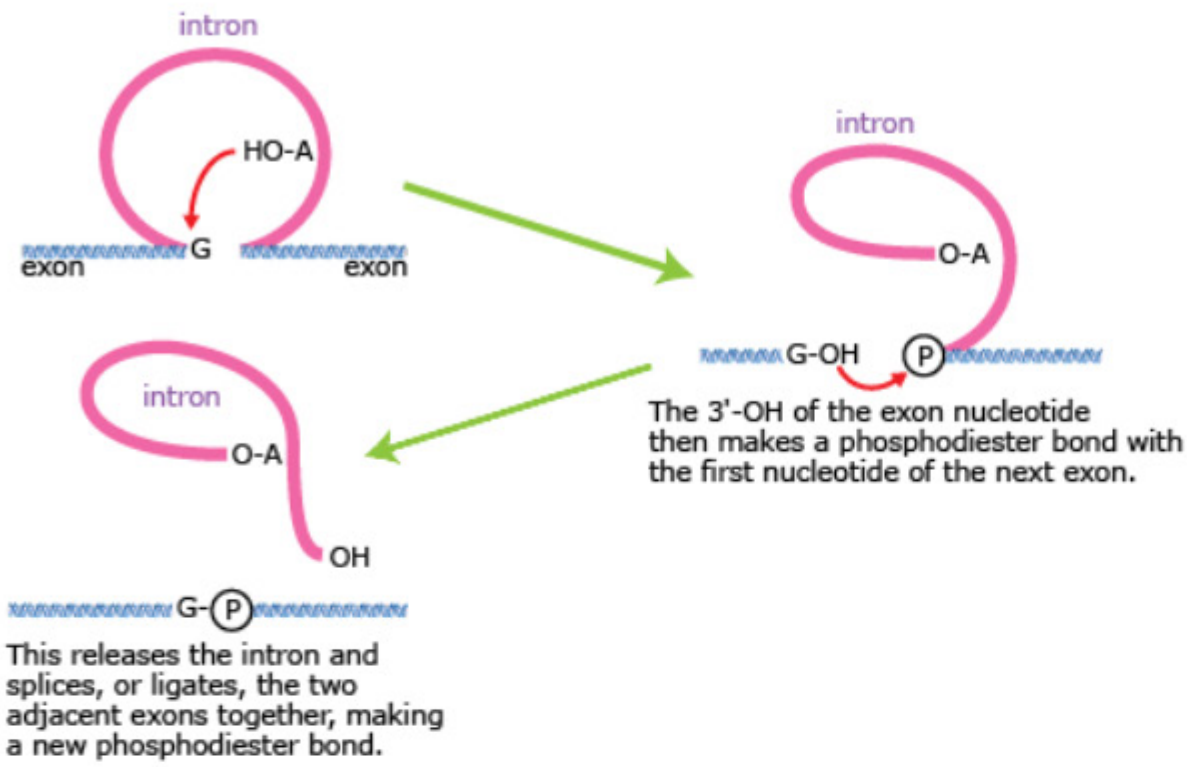
The splicing of introns occurs through a "lariat" or loop-like intermediate. The consequence is joining, or splicing, of the two adjacent exons with removal of the intervening intronic sequence.



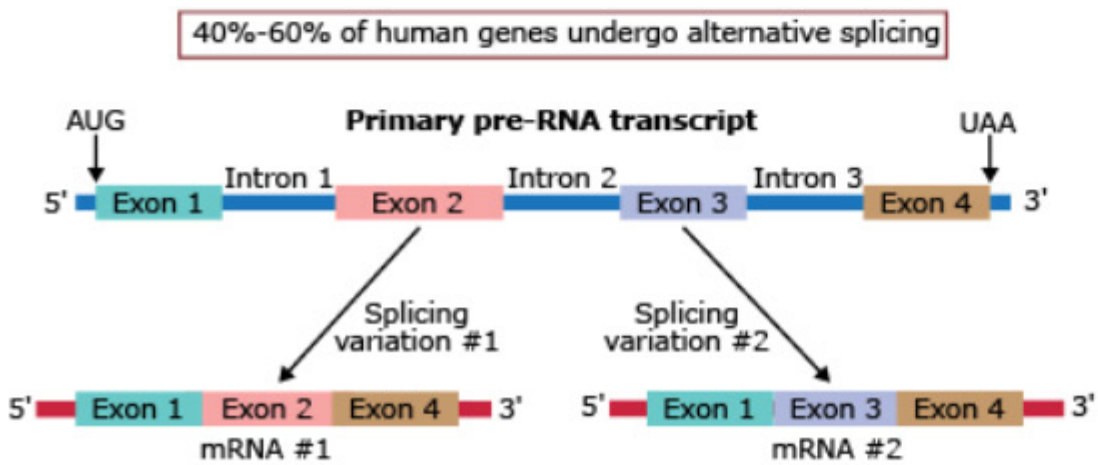
▲ **Figure 3–6.3B** "Lariat" Intermediate

Capping, polyadenylation, and splicing convert the hnRNA molecule to mRNA, which associates with proteins in the nucleus that transport it into the cytoplasm, where it can interact with the machinery required to translate it into proteins.

6.4 Alternative Processing of hnRNA

The information from the Human Genome Project suggests that human DNA contains about 20,000 genes (the genome). From this estimate, one might conclude that the total number of proteins in the human proteome would be somewhat less than 20,000 because some of these genes encode rRNA, tRNA, snRNA, and perhaps other types of RNA that are not translated to protein products. Evidence is not consistent with this view. The human proteome actually consists of perhaps 100,000 different proteins, raising the question: How can there be more proteins (100,000) than genes to encode them (20,000)?

6.4.1 Alternative Splicing



▲ **Figure 3–6.4A** Alternative Splicing

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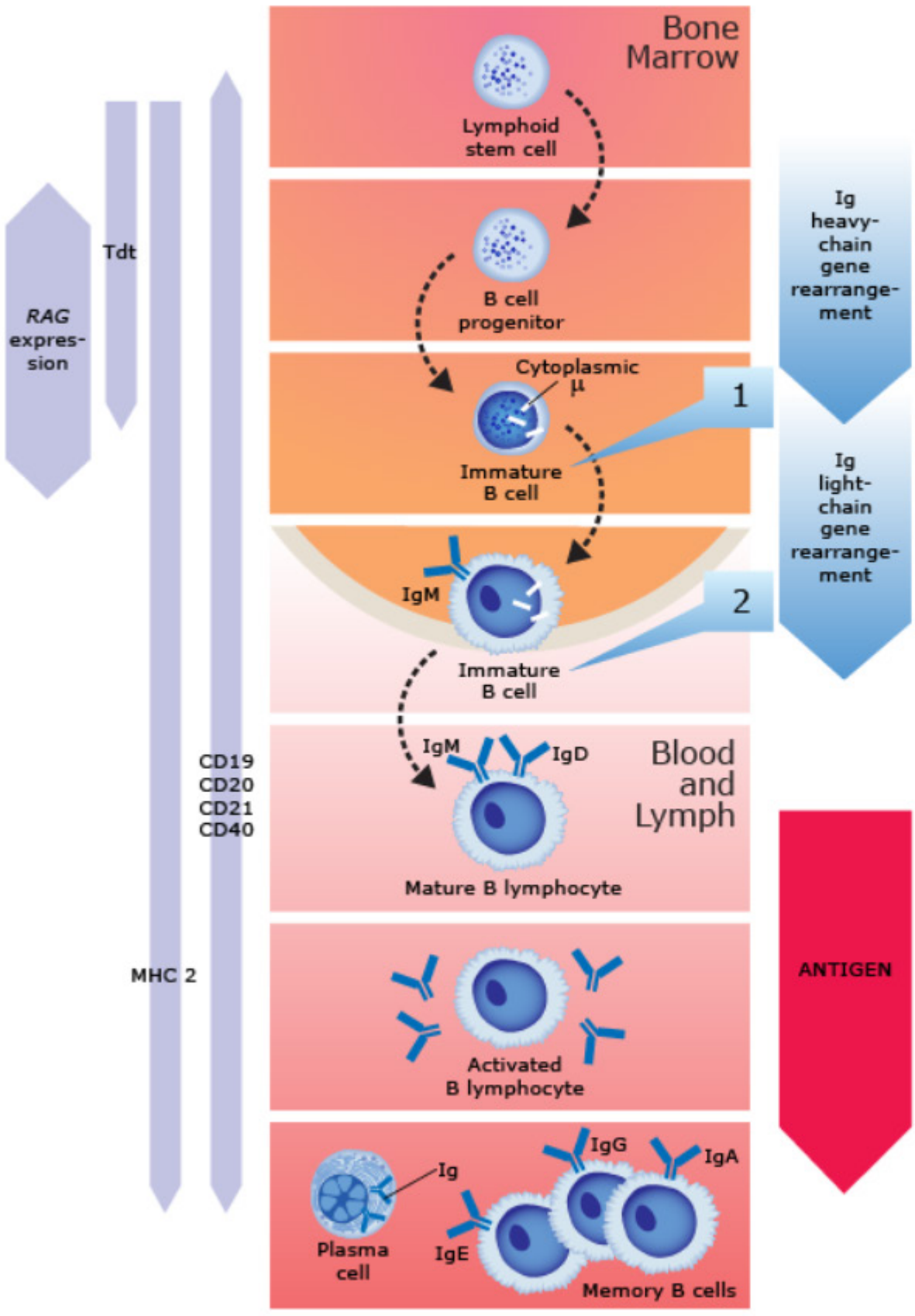
The primary transcripts of several genes in the human genome undergo alternative splicing either in the same cell/tissue, in different tissues, or at different developmental stages. One well-documented example is the expression of the transmembrane form of the antigen receptor (an antibody) and the secreted form of the same antibody lacking the transmembrane domain.



▲ **Figure 3–6.4B** Antigen Expression

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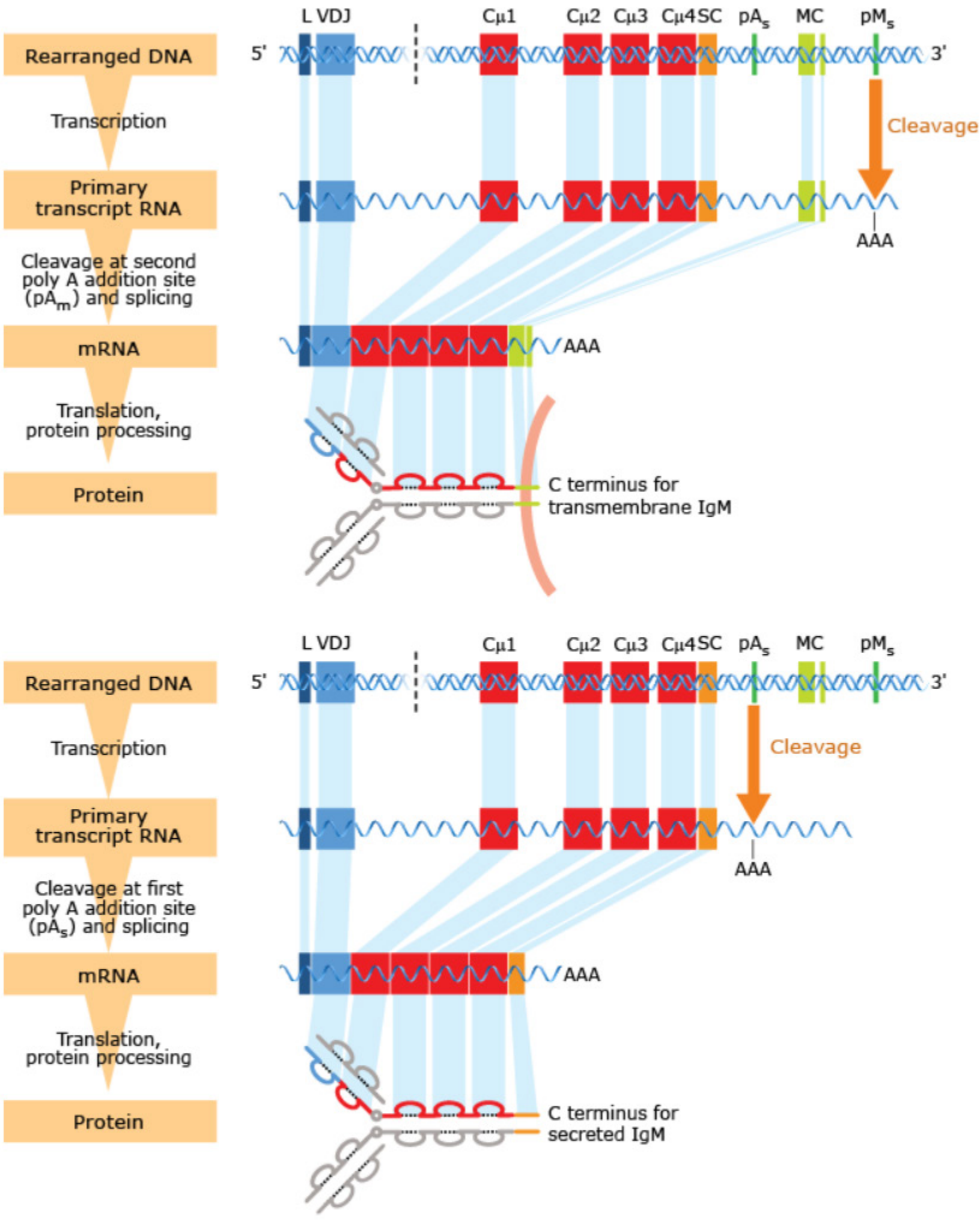

▲ **Figure 3–6.4C** Transmembrane and Secreted IgM

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B lymphocyte (B cell) maturation involves an example of alternative processing as well. In this case, the mature but as yet unstimulated B cell produces a primary transcript from the heavy-chain locus that has both the μ and the δ heavy-chain cassettes, each with their own termination signal. At this point in the B cell maturation, some of the primary transcripts terminate at the end of the μ poly-A site and the antibody produced is IgM. In another primary transcript, the Cμ cassette will be looped out with the previous intron and the resulting antibody is IgD. Both classes of antibody will retain the same antigen specificity.

6.4.2 Alternative Processing Is Not Random

Within any transcription unit, the versions of processing are not random. Only specific combinations of exons are allowed. The mechanism for this control is beyond the subject matter for the USMLE. Mutations that affect proper processing can cause genetic disease.

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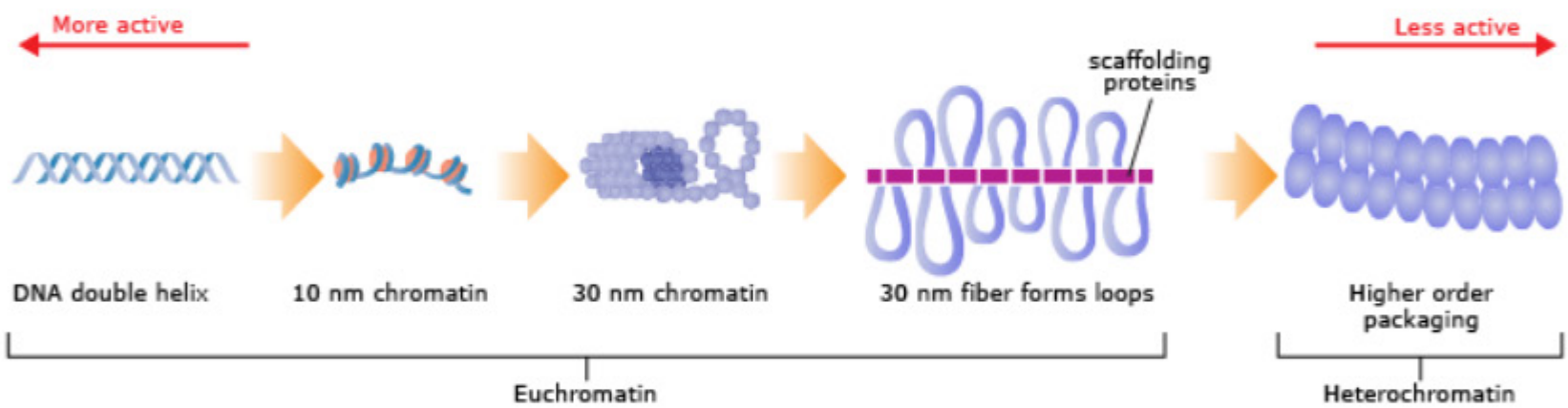
7 Control of Gene Expression at the Transcriptional Level

The magnitude of gene expression is one of the major mechanisms cells, tissues, and organisms employ to control the activity of their enzymes and the other components of their structure and metabolism. As enzymes and other essential proteins incur damage over time, they lose their activity and must be replaced by new, more functional copies. In like manner, as internal and external environments change, more substantial changes in the rates of individual metabolic pathways may be required. Hormone-mediated changes in the rates of transcription are among the factors that contribute to these responses.

7.1 Gaining Access to the Gene

Throughout interphase of the eukaryotic cell cycle, each chromosome is a mixture of *heterochromatin* (more condensed) and *euchromatin* (less condensed). Even the areas of euchromatin, however, have DNA packaged to some extent in nucleosomes (10 nm chromatin fibers and 30 nm chromatin fibers).



▲ **Figure 3–7.1** Euchromatin and Heterochromatin

In order to transcribe a gene, the DNA in the gene's location needs to be opened up and made available to RNA polymerase in a way that does not allow the DNA to be susceptible to nuclease digestion. This is accomplished through the action of transcription factors that bind reversibly to the histone proteins and DNA. These transcription factors encourage binding of coactivators, interact with the DNA to facilitate RNA polymerase binding, and protect the opened DNA from random nuclease attack. Three of the important modifications are shown in Table 3–7.1. These modifications are referred to as *epigenetic*—they reflect heritable changes to DNA structure that do not change the fundamental genetic sequence.

Important Concept

Epigenetics is the study of heritable changes to DNA structure that do not alter the underlying sequence. DNA methylation and histone modification are well-known examples.

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▼**Table 3–7.1** Epigenetic Modifications to Histone and DNA

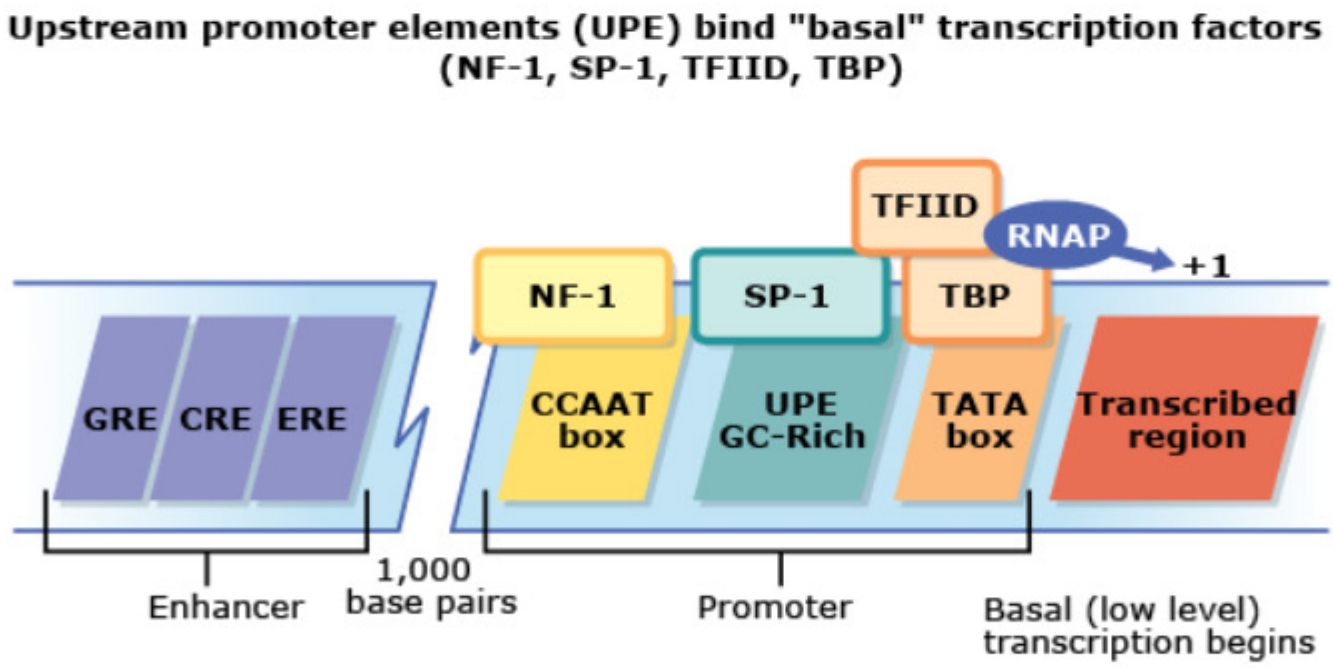
Modification	Effect on Gene Expression
Histone acetylation (lysine)	Increases gene expression
Histone deacetylation	Decreases gene expression
Histone methylation (lysine)	Decreases gene expression
Histone demethylation	Increases gene expression
DNA methylation (CpG) (5-Me-Cytosine)	Decreases or silences gene expression, often for long time periods (e.g., imprinting)

7.2 Transcription Factors and Their Binding Sites in the DNA

Once the DNA has been made available, the transcription factors bind to the DNA and form complexes that modify expression of one or more genes. There are two classes of transcription factors:

- *Activator proteins*, which bind to DNA sequences within the promoter region *(upstream promoter elements)* and other ones that bind at enhancer elements.
- *Repressor proteins*, which bind to DNA sequences named silencer elements.

In order for a relatively low level of transcription to be maintained (basal transcription), *general transcription factors* must bind to upstream promoter elements in the DNA. This sustains the rate of transcription necessary to replace damaged copies of the gene product (an enzyme, structural protein, etc.).



▲**Figure 3–7.2** General Transcription Factors

These general transcription factors form a complex that allows RNA polymerase to bind and initiate transcription. Many genes have binding sites for these factors. This allows one type of transcription factor to help maintain basal transcription of many genes with a common upstream promoter sequence recognized by this transcription factor.

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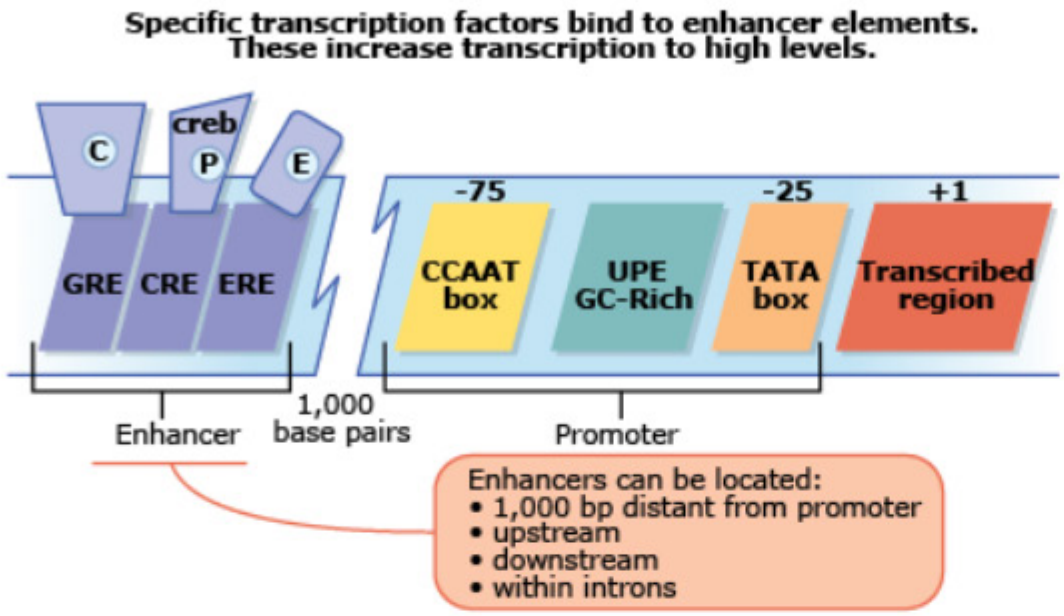
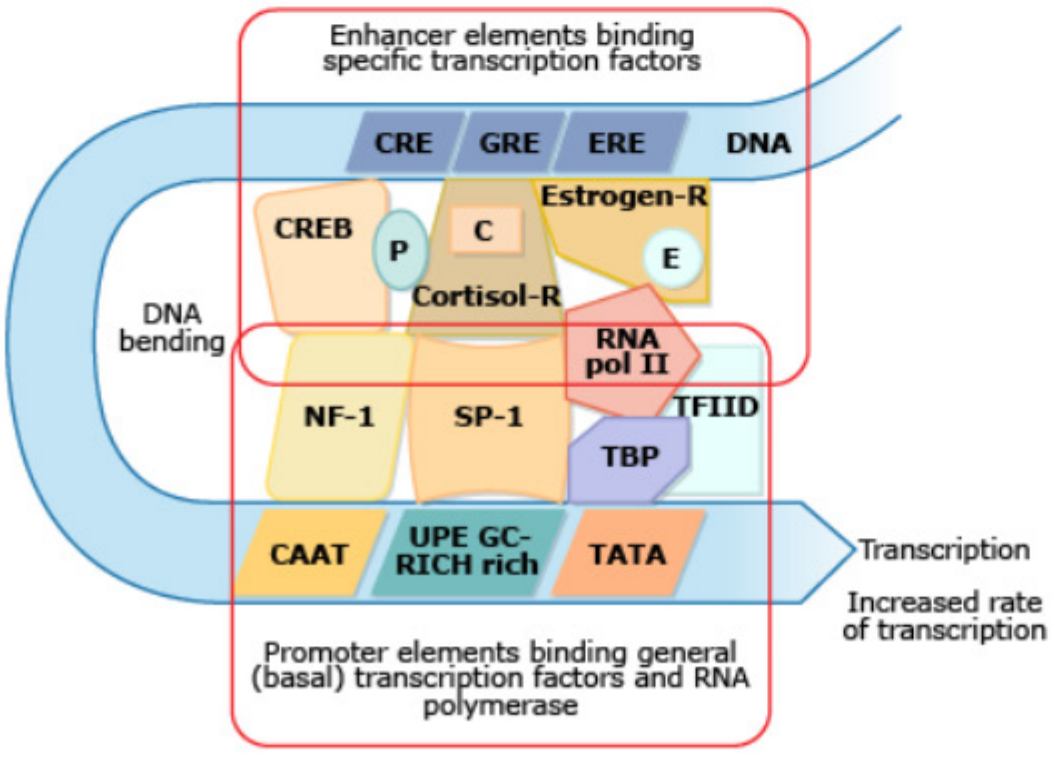
7.3 Special Transcription Factors

Specific transcription factors mediate a much higher level of transcription in response to specific signals such as hormones, growth factors, or developmental cues.



▲ **Figure 3–7.3A** Special Transcription Factors

Specific transcription factors do not bind within the promoter. Their binding sites in the DNA are named *enhancer elements* and are often at somewhat remote sites upstream or downstream from the gene whose expression they control. They also may be found within an intron of the gene. Relevant enhancer elements are often grouped together in the DNA. The entire group is referred to as an enhancer, which contains elements, each of which are binding sites for different transcription factors (activator proteins). Through bending of the DNA, the components of the transcription complex come together to generate a much higher rate of transcription.



▲ **Figure 3–7.3B** Enhancer Elements

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▼Table 3–7.3 Specific Transcription Factors

Transcription Factor	Enhancer Element	Examples of Functions
Steroid Receptors (Zinc Finger Proteins)	HRE (ERE, GRE)	GRE: Increase transcription of PEPCK gene stimulating gluconeogenesis in hepatocytes
Vitamin D Receptors (VDR) (Zinc Finger Proteins)	VDE	• Increase Ca2+ uptake in intestine and decrease Ca2+ excretion • Stimulate osteoblasts, which stimulate pre-osteoclast differentiation (releases Ca2+) • Act, with Retinoid X Receptor (RAX and RAR), as growth regulator in many cells
Retinoid and Retinol Receptors (Zinc Finger Proteins)	RXRE	• Growth and differentiation of many cell types • *Major exam point: all-trans retinoic acid used in AML to enforce proper differentiation of neutrophils* • RXR also plays a role in reproduction, cellular differentiation, bone development, hematopoiesis, and pattern formation during embryogenesis
cAMP Response Element Binding Protein (CREB) (Leucine Zipper Proteins)	CRE	Modulates transcription based on cAMP levels. Protein kinase phosphorylates and activates CREB in response to water-soluble hormones such as glucagon and epinephrine.
Peroxisome Proliferator Activated Receptors (PPARα and PPARγ) (Zinc Finger Proteins)	PPRE	Control expression of many genes in lipid metabolism. Normal ligands are fatty acids or prostaglandins. Xenophobic ligands include: • Fibrates (lower TGL) • Thiazolidinediones (stimulate insulin-responsive genes)
NFκB	κB	Controls expression of many genes for proteins within the immune system (e.g., the κ light gene)
JAK-STAT JAK: Janus kinase (just another kinase) STAT: Signal transducers and activators of transcription	CRE	Tyrosine kinase is important for mediating signals from many cytokines. Similar but not identical to the IRS proteins.
• **Pax 3 protein** • **Sonic hedgehog protein** • **HOX gene proteins**		Transcription factors controlling gene expression during development in utero. Loss of function mutations may cause abnormal development: • Waardenburg syndrome (Pax 3 gene) • Holoprosencephaly and loss of proper midline development (sonic hedgehog gene)

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Clinical Application

Waardenburg Syndrome

Type 1: Mutation in PAX3 gene

- Pigmentary abnormalities (white forelock, heterochromia iridis, patchy hypopigmentation of skin)
- Sensorineural hearing loss
- Dystopia canthorum
- No limb abnormalities

Type 2: Similar to Type 1, but also upper limb abnormalities

During embryonic development, the PAX3 gene is active in cells called neural crest cells. These cells migrate from the developing spinal cord to specific regions in the embryo, directing differentiation of neural crest cells to form specialized tissues, as well as playing an important role in early myogenesis. In addition to its role in the formation of tissues and organs, the PAX family of transcription factors is also important for maintaining the normal function of certain cells after birth.

Failure of Complete Midline Development: Mutation in Sonic Hedgehog Gene

- Holoprosencephaly: Failure of forebrain of the embryo to separate into two hemsipheres
- Defects in the face
- Most children survive only a few days
- A minority are less severely affected

Ralph C. Eagle, Jr./Science Source

▲ **Figure 3–7.3C** Holoprosencephaly

Sonic hedgehog (SHH) is one of three proteins in the mammalian signaling pathway family called hedgehog, and is encoded by the SHH gene. SHH plays a key role in regulating vertebrate organogenesis, such as in the growth of digits on limbs and organization of the brain. SHH diffuses in the embryo to form a concentration gradient that has different effects on the cells of the developing embryo depending on its concentration.

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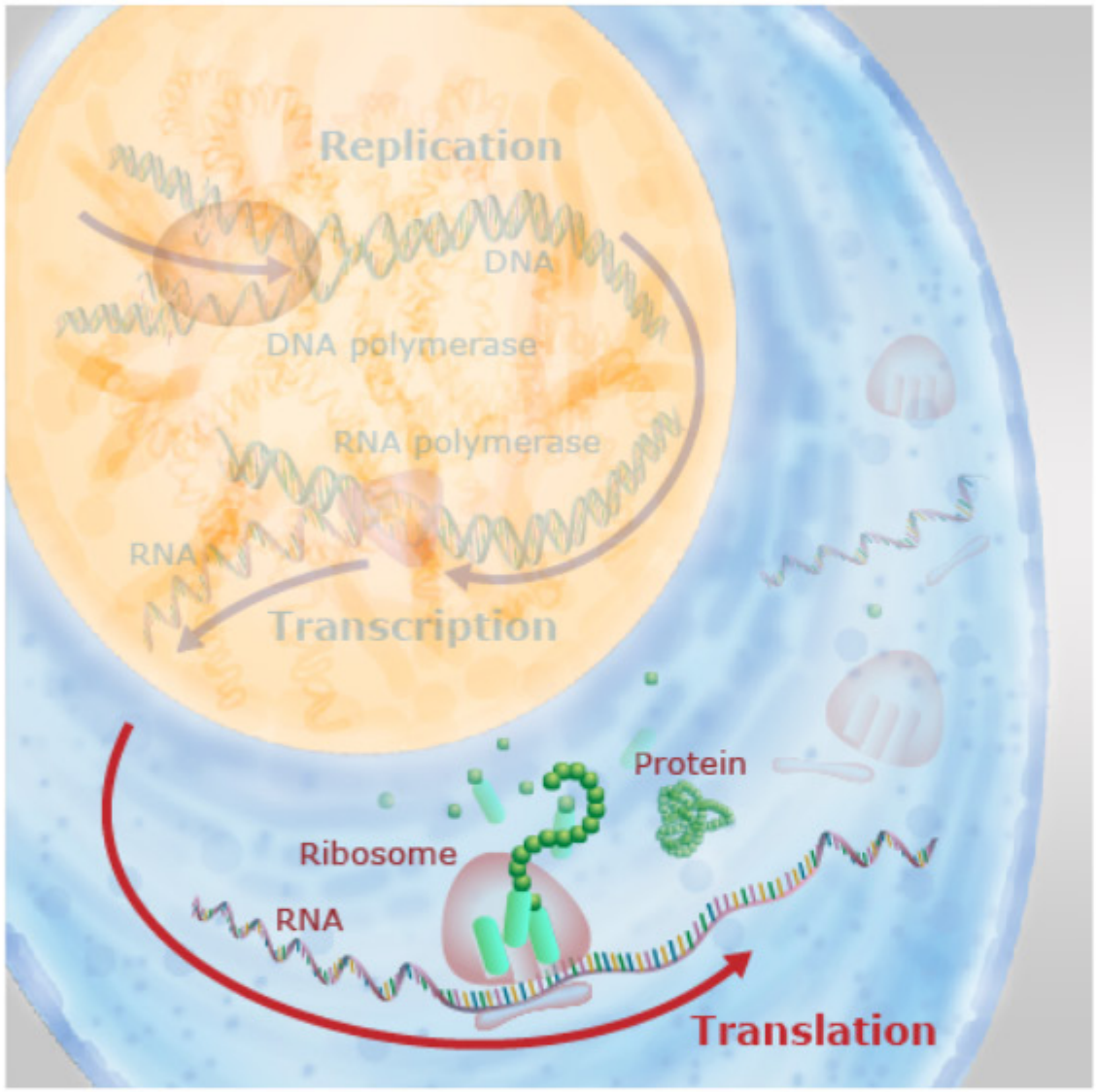
Eukaryotic Gene Expression: Translation

1 Overview of Translation



▲ **Figure 4–1.0** Translation

USMLE® Key Concepts

For Step 1, you must be able to:

- Describe the basic process of translation and the effects of important toxins and antibiotics on the process.
- Explain the hierarchy of protein structure and cellular mechanisms for posttranslational modifications.
- Identify post-translational targeting strategies, especially for transmembrane proteins, secreted proteins, and lysosomal enzymes.
- Name diseases related to defects in co- and posttranslational processes.

2 Requirements for Protein Synthesis

- Fully processed mRNA in the cytoplasm.
- Ribosomes.
- Sufficient quantities of all the 20 amino acids.
- An energy source.
- *Initiation factors* (eIF), *elongation factors* (eEF), and *termination factors* (eTF), all of which are proteins that facilitate events on the ribosome during protein synthesis. The comparable factors in the prokaryotic cells are designated without the lowercase "e."

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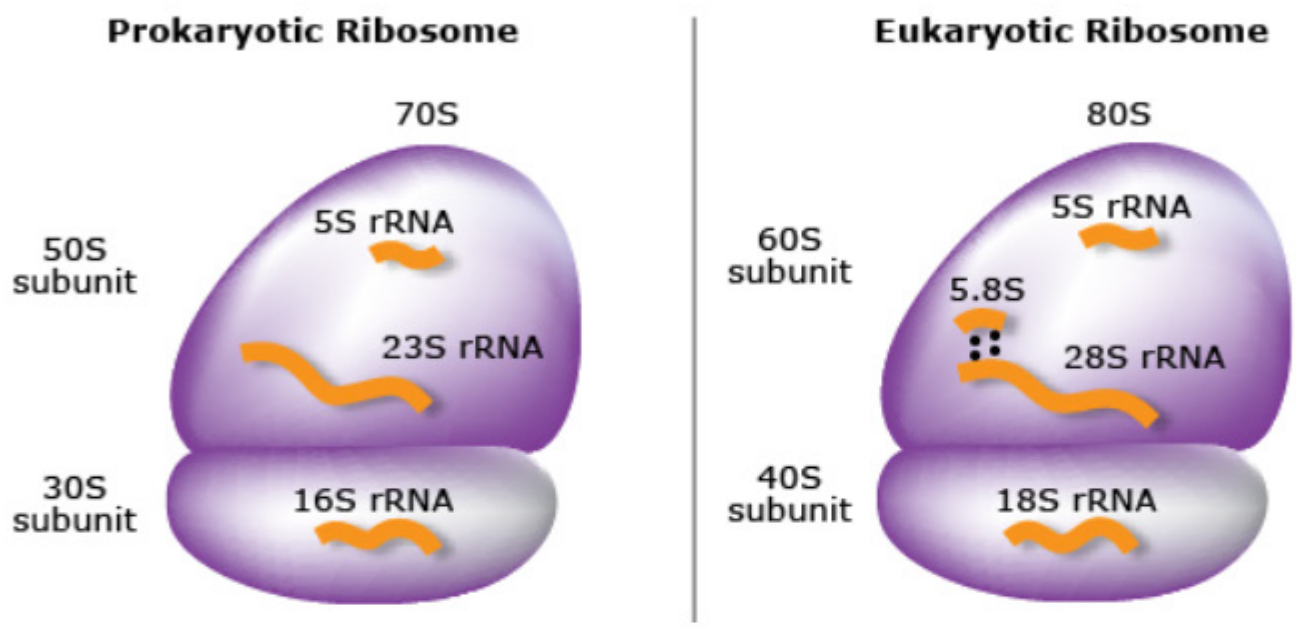
3 Ribosomes

Ribosomes are cytoplasmic structures composed of ribosomal rRNA and protein. All but one of the rRNAs are encoded by a single gene, producing an RNA that is subsequently cut by *RNases* to yield the different sizes of rRNA (28S, 5.8S, and 18S). These rRNAs bind with ribosomal proteins to form the large and small subunits of the ribosome. This all happens in the nucleolus of eukaryotic cells. *The only rRNA not transcribed in the nucleolus by RNA polymerase I is the 5S rRNA, which is produced by RNA polymerase III in the nucleoplasm.*

Important Concept

- RNA polymerase I: rRNAs *except for the 5S rRNA*
- RNA polymerase II: pre-mRNA, mRNA, and snRNA for all spliceosomes
- RNA polymerase III: tRNAs and the *5S rRNA*



▲ **Figure 4–3.0** Prokaryotic and Eukaryotic Ribosomes

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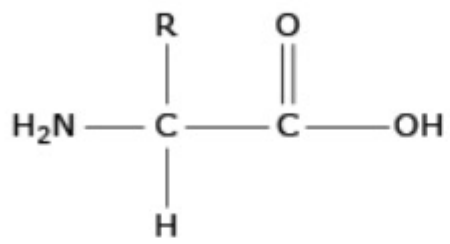
4 Amino Acids

4.1 Amino Acid Structure

Proteins are polymers of amino acids, which consist of four elements:

- Central, or alpha carbon
- Amino group
- Carboxylic acid group
- A side chain (R group)



▲ **Figure 4–4.1** Amino Acid Structure

The side chains (R groups) are different molecular structures that give each amino acid unique structural and functional properties. Proteins include 20 different amino acids, each of which has a unique side chain. The amino acids are categorized based on the structural and chemical properties of their side chains. There are four different groups: (1) *aliphatic* amino acids, (2) *aromatic* amino acids, (3) *polar* amino acids, and (4) *charged* amino acids.

4.1.1 Aliphatic Amino Acids

Aliphatic amino acids are *non-polar* and *hydrophobic*. The side chains are hydrocarbon chains with no polar, charged, or other reactive groups. Because they are hydrophobic, they are usually *found on the interior of proteins or in lipid bilayers* where they associate with other hydrophobic molecules rather than on the surface interacting with water.

The aliphatic amino acids (with their corresponding three- and one-letter codes) are:

- Glycine (Gly or G)
- Alanine (Ala or A)
- Valine (Val or V)
- Leucine (Leu or L)
- Isoleucine (Ile or I)
- Proline (Pro or P)

4.1.2 Aromatic Amino Acids

These amino acids contain some form of benzene ring in their side chain structure. They are *large* and *bulky* and thus occupy considerable space in protein structure. They are generally hydrophobic, although tryptophan and tyrosine are made less so by virtue of attached amino or hydroxyl groups.

The aromatic amino acids are:

- Phenylalanine (Phe or F)
- Tyrosine (Tyr or Y)
- Tryptophan (Trp or W)

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4.1.3 Polar Amino Acids

These have side chains with polar amino, hydroxyl, or sulfhydryl groups. These polar groups make them *hydrophilic* and, consequently, they generally are *found on the surface of proteins*. These polar groups are chemically reactive and may form intra- or inter-peptide covalent bonds (like sulfhydryl bonds), attach to protein modifiers (as in phosphorylation or glycosylation), or participate in catalysis in enzymes.

The polar amino acids are:

- Serine (Ser or S)
- Threonine (Thr or T)
- Cysteine (Cys or C)
- Methionine (Met or M)
- Aspargine (Asp or N)
- Glutamine (Gln or Q)

4.1.4 Charged Amino Acids

These have side chains that include a chemical group that is either positively (basic) or negatively (acidic) charged at neutral pH. These are *hydrophilic* and may be *found on the surface of proteins*. Due to their charged character, these amino acids often form intra- or inter-peptide salt bridges that help determine protein structure. These groups are chemically reactive and are often involved the catalytic reactions of enzymes.

The negatively charged (acidic) amino acids are:

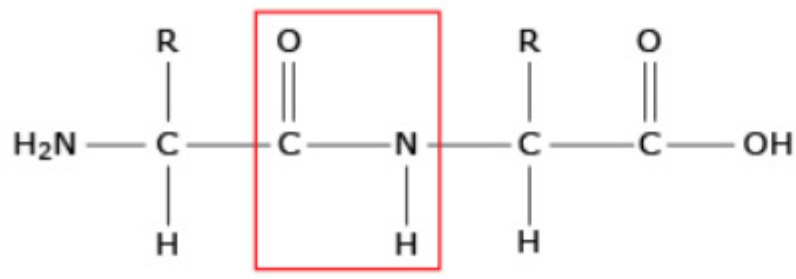
- Aspartate (Asp or D)
- Glutamate (Glu or E)

The positively charged (basic) amino acids are:

- Arginine (Arg or R)
- Lysine (Lys or K)
- Histidine (His or H)

4.2 Polypeptides

Proteins are polymers of amino acids, also termed polypeptides. The amino acids are linked by covalent bonds, called *peptide bonds,* between the amino group of one amino acid and the carboxylic acid group of another. This creates polarity in proteins; every polypeptide has a free amino group (the *amino-* or *N-terminus*) and a free carboxylic acid group (the *carboxy-* or *C-terminus*).



▲ **Figure 4–4.2** Peptide Bond

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4.3 Essential Amino Acids

In order to carry out translation to make a protein, all 20 of the common amino acids must be present in sufficient quantities in the cytoplasm. Ten of the amino acids can normally be synthesized by humans. The other 10 must be acquired from the diet. These *10 essential amino acids* are:

- Histidine
- Methionine
- Threonine
- Valine
- Isoleucine
- Phenylalanine
- Tryptophan
- Leucine
- Lysine
- Arginine

Arginine is considered to be a semi-essential amino acid. It is not essential in healthy adults, but is essential during periods of positive nitrogen balance, as in childhood, pregnancy, or recovery from periods of substantial injury. If even one of these essential amino acids is not present, or not present in sufficient quantity to meet the body's needs, protein synthesis will be decreased or, in extreme cases, will not occur.

Important Concept

Kwashiorkor, severe protein malnutrition, is an example of a state in which arginine would become an essential amino acid.

4.4 Genetic Code Table

5'	U		C		A		G		3'
U	UUU	*Phe*	UCU	*Ser*	UAU	*Tyr*	UGU	*Cys*	U
	UUC		UCC		UAC		UGC		C
	UUA	*Leu*	UCA		UAA	*Stop*	UGA	*Stop*	A
	UUG		UCG		UAG		UGG	*Trp*	G
C	CUU	*Leu*	CCU	*Pro*	CAU	*His*	CGU	*Arg*	U
	CUC		CCC		CAC		CGC		C
	CUA		CCA		CAA	*Gln*	CGA		A
	CUG		CCG		CAG		CGG		G
A	AUU	*Ile*	ACU	*Thr*	AAU	*Asn*	AGU	*Ser*	U
	AUC		ACC		AAC		AGC		C
	AUA		ACA		AAA	*Lys*	AGA	*Arg*	A
	AUG	*Met*	ACG		AAG		AGG		G
G	GUU	*Val*	GCU	*Ala*	GAU	*Asp*	GGU	*Gly*	U
	GUC		GCC		GAC		GGC		C
	GUA		GCA		GAA	*Glu*	GGA		A
	GUG		GCG		GAG		GGG		G

▲ **Figure 4–4.4** Codon Table

You do not have to memorize the codon table, but it saves time if you know the three stop codons (see Table 4–4.4) and the start codon.

▼ **Table 4–4.4** Stop Codons

In the mRNA	Mnemonic	In the DNA Coding Strand
UGA	U Go Away	TGA
UAG	U Are Gone	TAG
UAA	U Are Away	TAA

None of the stop codons specify an amino acid. They just mark the stop site for translation in mRNA.

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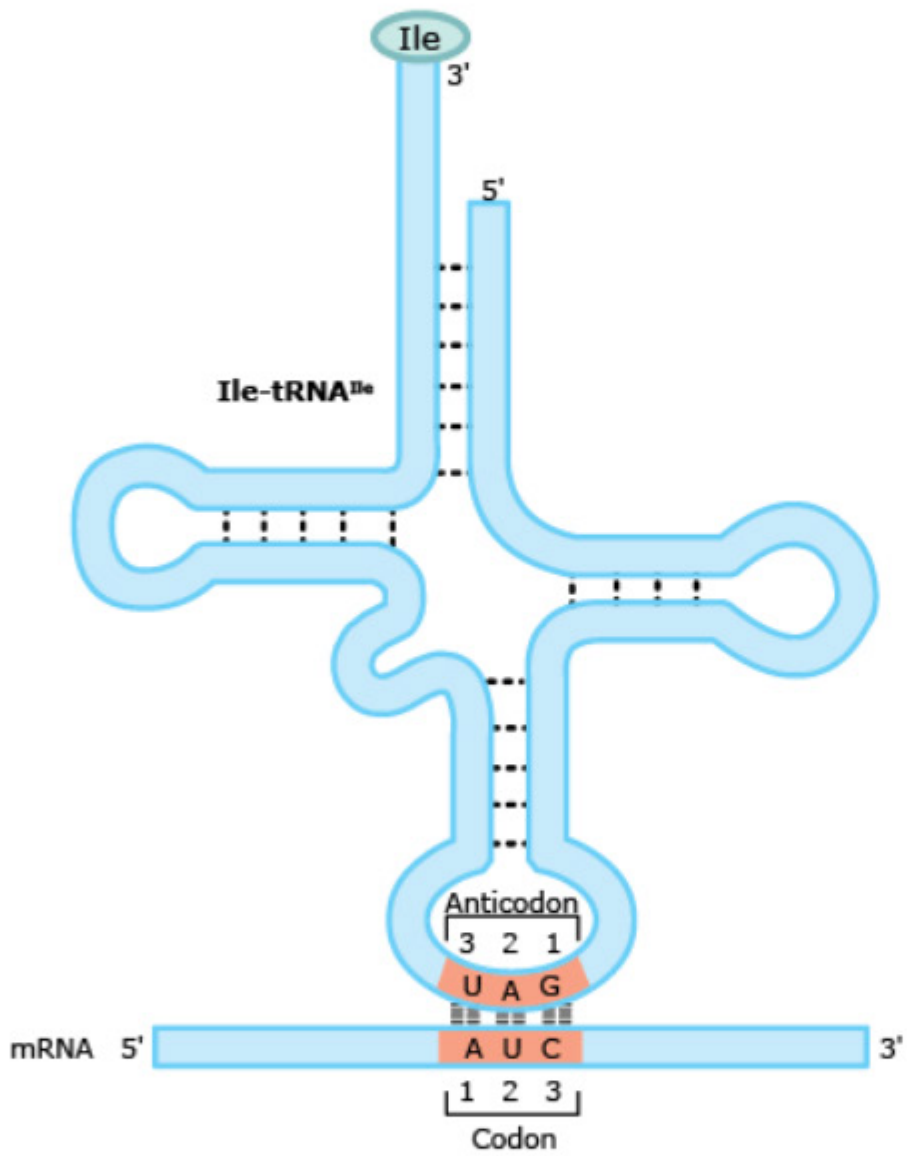
In contrast, the codon AUG (ATG in the coding strand of the gene) is the "start" codon for translation to begin. The codon AUG also specifies the amino acid methionine.

4.5 Amino Acid Activation and Transfer RNA (tRNA)

There are many different tRNAs, each specific for one of the 20 amino acids used to produce proteins. Because there are 61 codons, many codons specify for more than one amino acid, a property known as redundancy.

The other aspect of the tRNAs is the ability to bind to the codon in the mRNA that specifies the amino acid carried by that tRNA. Figure 4–4.5A illustrates this concept.



▲ **Figure 4–4.5A** Base Pairing of Aminoacyl-tRNA With Codon in mRNA

The correct joining of a tRNA to its cognate amino acid is catalyzed by a class of enzymes named *aminoacyl tRNA synthetases*. The energy source for this reaction is the breakage of two high-energy bonds from an ATP. Once this reaction has occurred, the amino acid is said to be "activated" and the tRNA has been "charged" with the amino acid. It is now ready to participate in translation.

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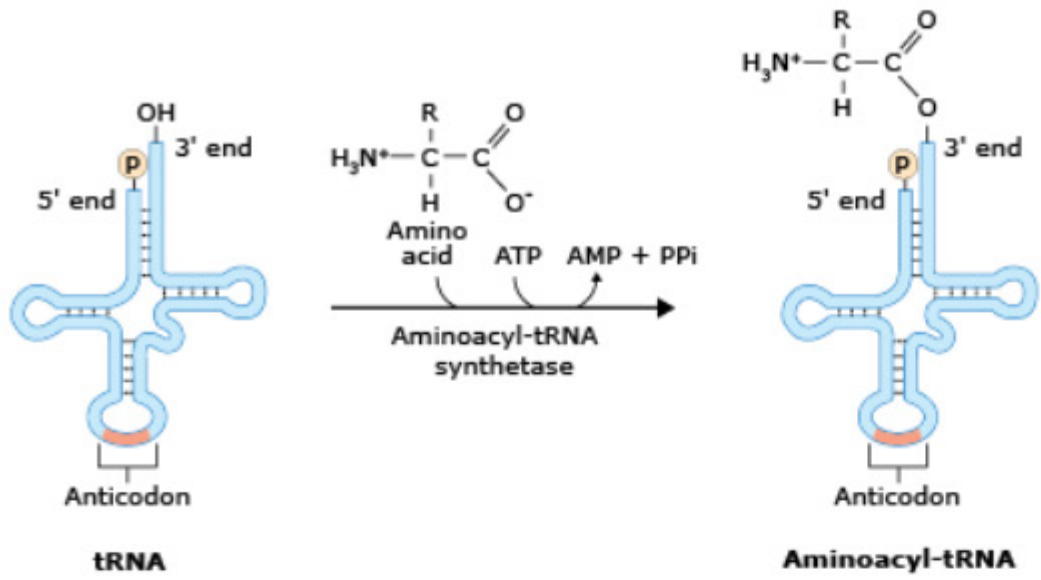

▲ **Figure 4–4.5B** Activation of Amino Acid for Protein Synthesis

Figure 4–4.5B shows a generic version of amino acid activation. A specific example would be:

$$\text{Lysine} + \text{tRNA}^{\text{lys}} + \text{ATP} \xrightarrow{\textit{Lysyl-tRNA synthetase}} \text{Lysyl-tRNA}^{\text{lys}} + \text{AMP} + 2\ \text{Pi}$$

Importantly, all of the *aminoacyl-tRNA synthetases proofread*. Before the enzyme releases the aminoacyl-tRNA, it makes a final check that the correct amino acid has been added. If the amino acid is not correct, the synthetase removes it. The proofreading is necessary because if the synthetase did not detect the error, there is nothing to catch it later on the ribosome.

4.6 Translation Factors

At certain points in translation, factors are required to assist the process. There are many of these factors (usually proteins) and they are designated according to the stage of translation for which they are required. For instance, eIF-1 is eukaryotic (e) initiation factor one. Therefore, it is a eukaryotic factor needed to form the initiation complex. For the most part, you will not need to know these individual factors, but in certain cases they are of importance in Step 1. As we review translation, such important factors will be identified.

In addition, in prokaryotic cells, several antibiotics target bacterial translation. Some are bacteriostatic and some are bactericidal.

▼ **Table 4–4.6** Antibiotics and Toxins That Inhibit Translation

Antibiotic	Process Inhibited	Site of Action
Streptomycin	Initiation complex binding	Prokaryotic – 30S
Tetracyclines	tRNA binding	Prokaryotic – 30S
Chloramphenicol	Peptidyl transferase	Prokaryotic – 50S
Macrolides	Translocation	Prokaryotic – 50S
Toxin	**Process Inhibited**	**Site of Action**
Shiga toxin/ricin	tRNA binding	Eukaryotic – 60S
Diphtheria toxin	Translocation	Eukaryotic – EF-2
Cycloheximide	Peptidyl transferase	Eukaryotic – 60S

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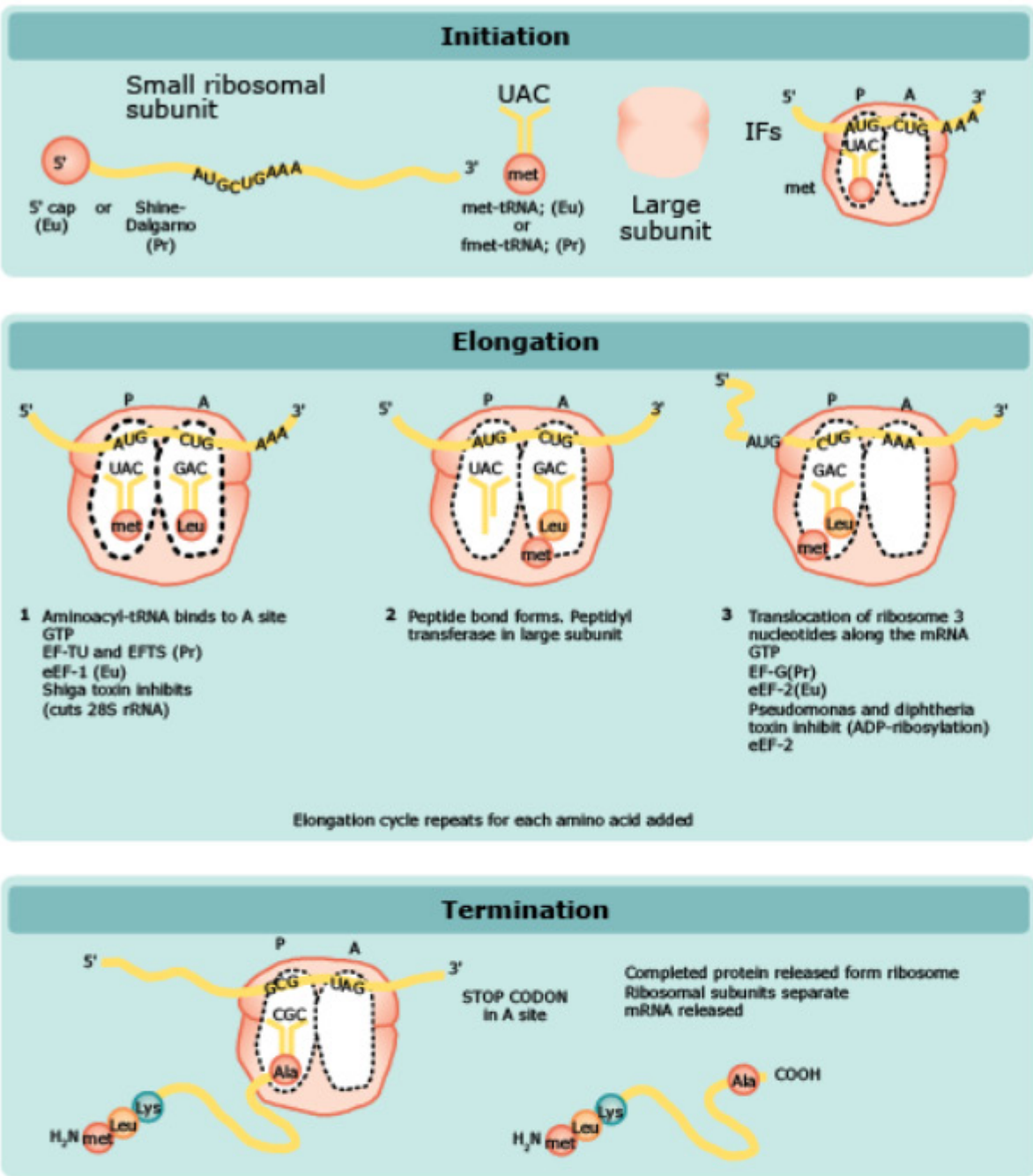
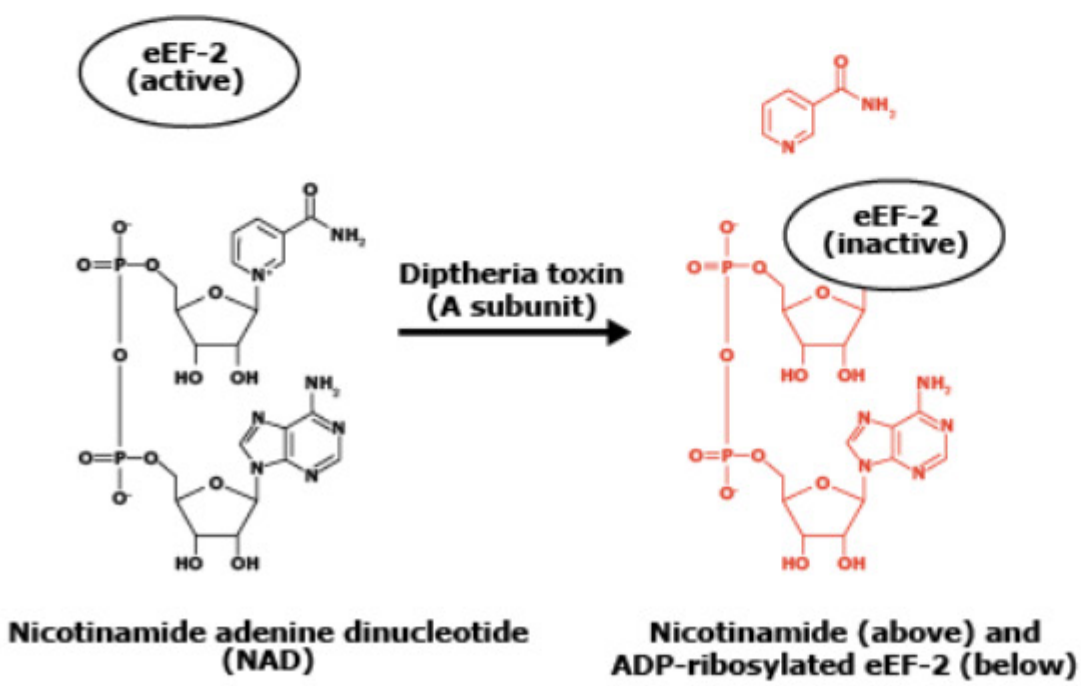


▲ **Figure 4–4.6A** Translation: Initiation, Elongation, and Termination



▲ **Figure 4–4.6B** ADP-Ribosylation

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5 Protein Folding, Posttranslational Modifications, and Targeting

One of the first modifications to newly synthesized proteins is proper *folding*. Strictly speaking, folding is a cotranslational modification that begins as the protein is elongated on the ribosome and is only completed after the protein is released from the ribosome. This process is influenced by the primary structure (amino acid sequence) of the protein and glycosylation (addition of sugar residues to amino acid side chains).

5.1 Protein Conformation

The function of each protein is determined by its *conformation:* the three-dimensional shape that is unique to every protein. Conformation is determined by structural interactions at four different levels: primary, secondary, tertiary, and quaternary.

5.1.1 Primary Structure

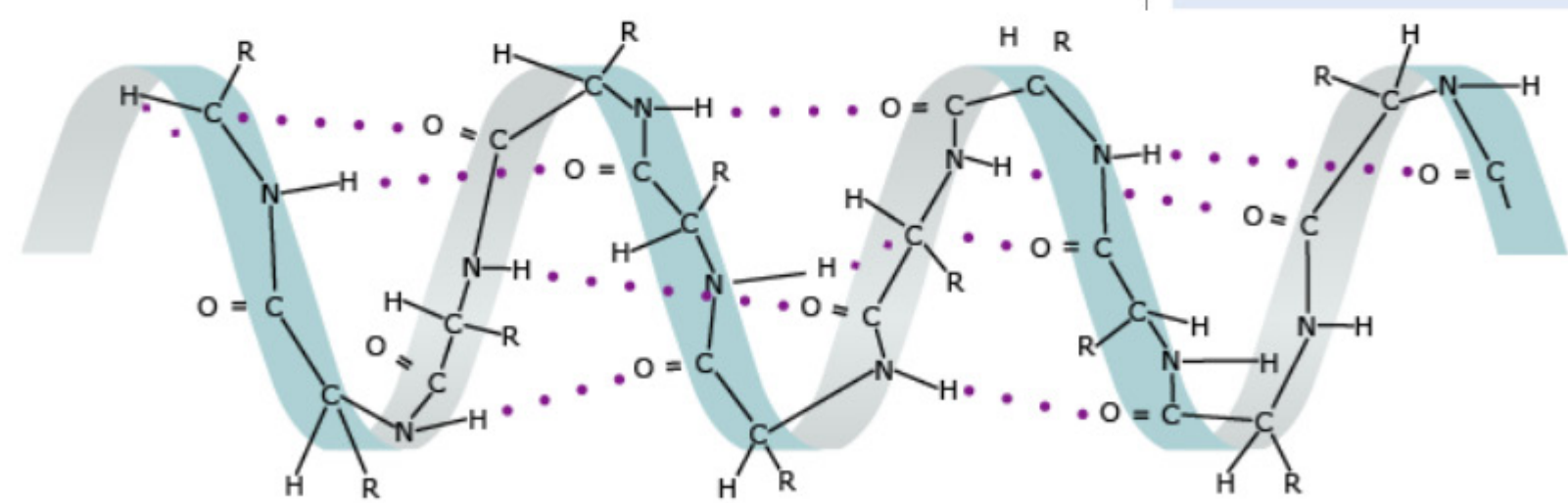
The primary structure is the protein sequence, the order of amino acids. This is determined by the DNA sequence in which the protein is encoded.

5.1.2 Secondary Structure

The secondary structure is composed of structural elements formed by hydrogen bonds between the backbone amino and carboxyl groups of amino acids. α helices form a spiral of amino acids, resulting in a peptide column. β-pleated sheets are multiple peptide stretches forming a sheet-like structure.

Clinical Application

Domains of a protein are formed when the protein begins to fold during and after translation. The domains often represent areas of the protein that have a specific function associated with them. For instance, the heavy chain of an IgG antibody begins with an antigen-binding domain at the N-terminus. This domain is encoded by the first exon of the antibody heavy-chain gene (VDJ) region. The last exon encodes the final carboxyl terminal domain. When bacteria, viruses, or toxins are bound by the antigen-binding domain, the FC portion of the antibody can bind to Fc-receptors on polymorphonuclear neutrophilic (PMN) leucocytes or on macrophages, which will ingest antibody-coated bacteria and kill them.



Hydrogen bonds between amino acids at different locations in polypeptide chain

▲ **Figure 4–5.1A** α Helix

Clinical Application

Aggregates of β-pleated sheets are responsible for Alzheimer's disease and amyloidosis.

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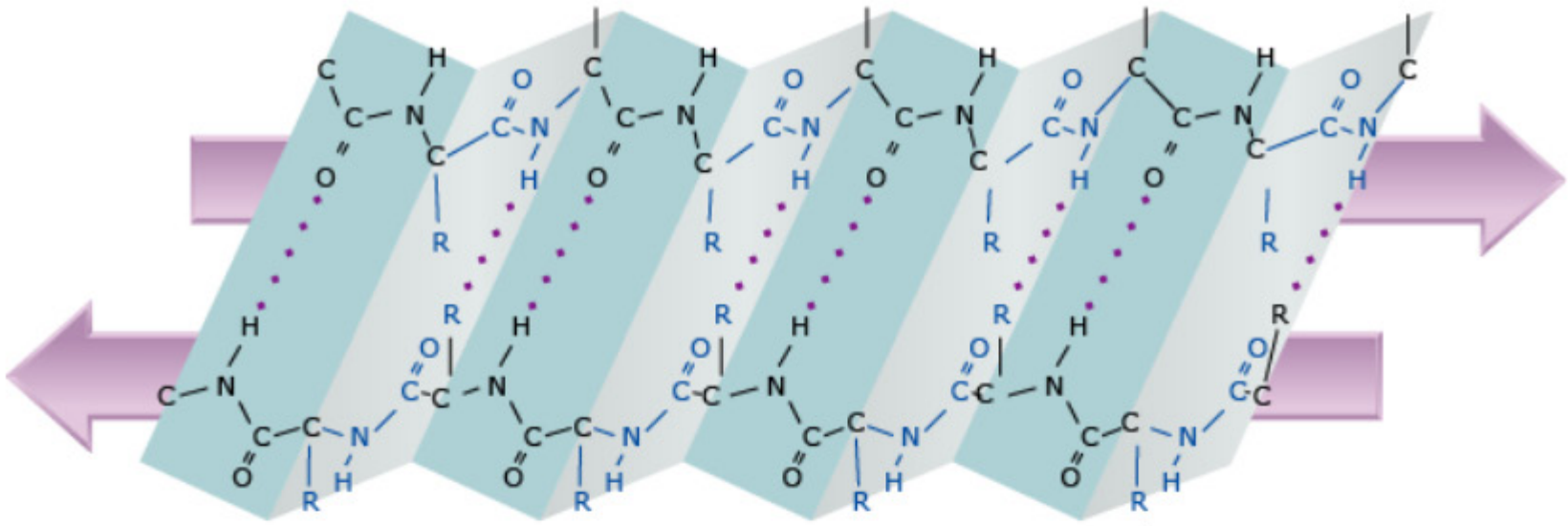
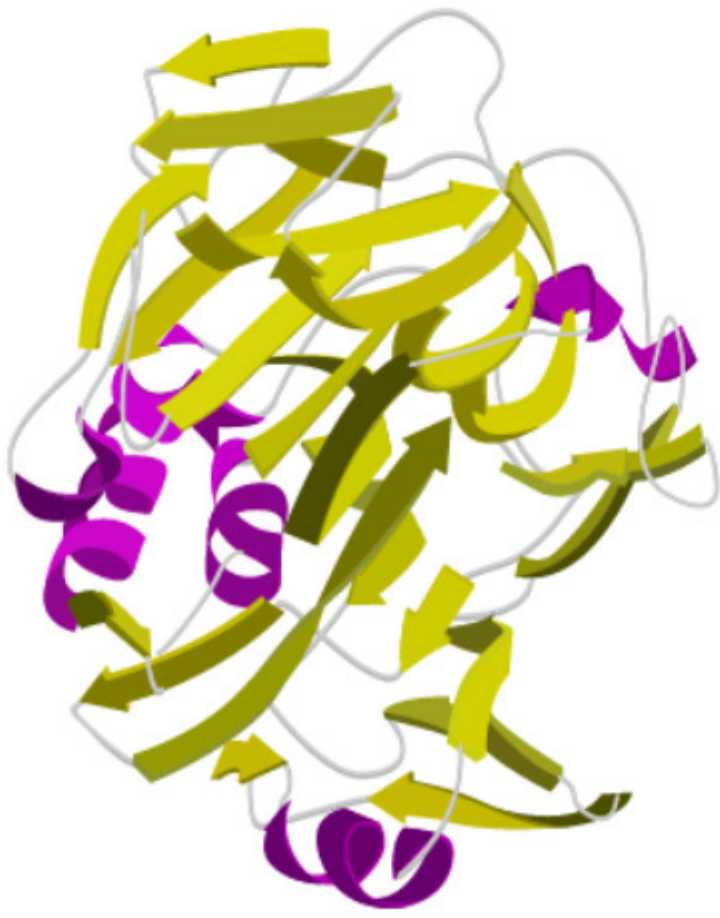


▲ **Figure 4–5.1B** β-Pleated Sheets

5.1.3 Tertiary Structure

Tertiary structure consists of the actual three-dimensional form that a polypeptide assumes after the secondary structure is in place. This is determined by a number of different forces or interactions:

- Covalent bonds between side chains (e.g., disulfide bonds between two cysteine amino acids)
- Ionic bonds between oppositely charged amino acids
- Hydrogen bonds between polar side chains and/or backbone amino and carboxyl groups
- Hydrophobic interactions between aliphatic amino acids.



▲ **Figure 4–5.1C** Tertiary Structure

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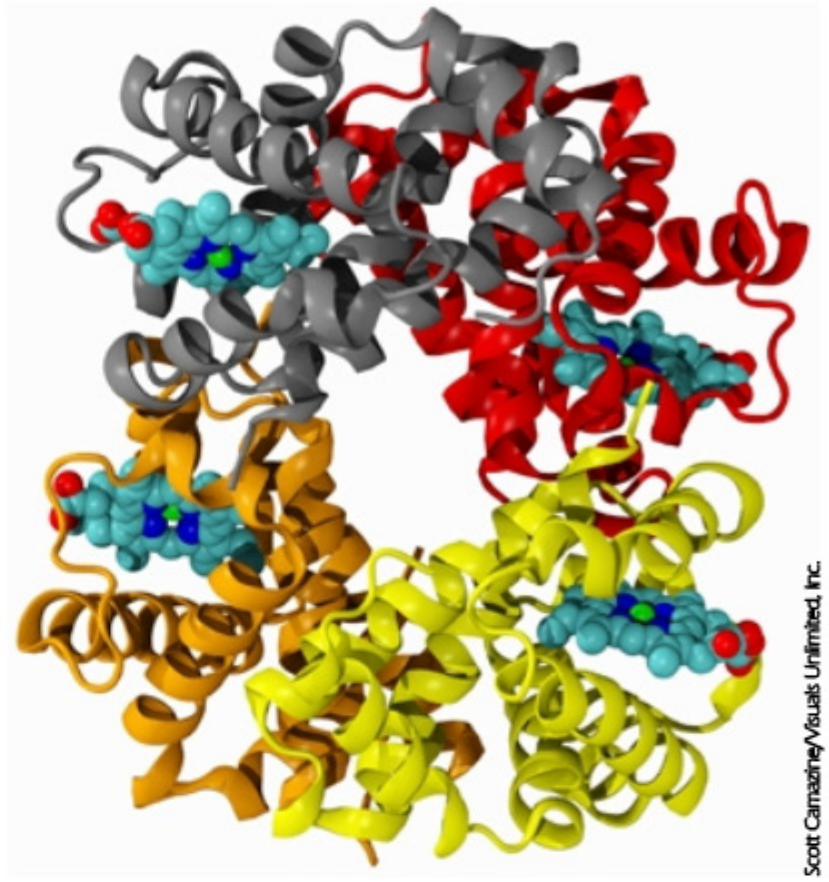
5.1.4 Quaternary Structure

Quaternary structure refers to interactions among two or more polypeptides to form multiprotein complexes. The forces that determine the interactions among these proteins are the same as those in tertiary structure.

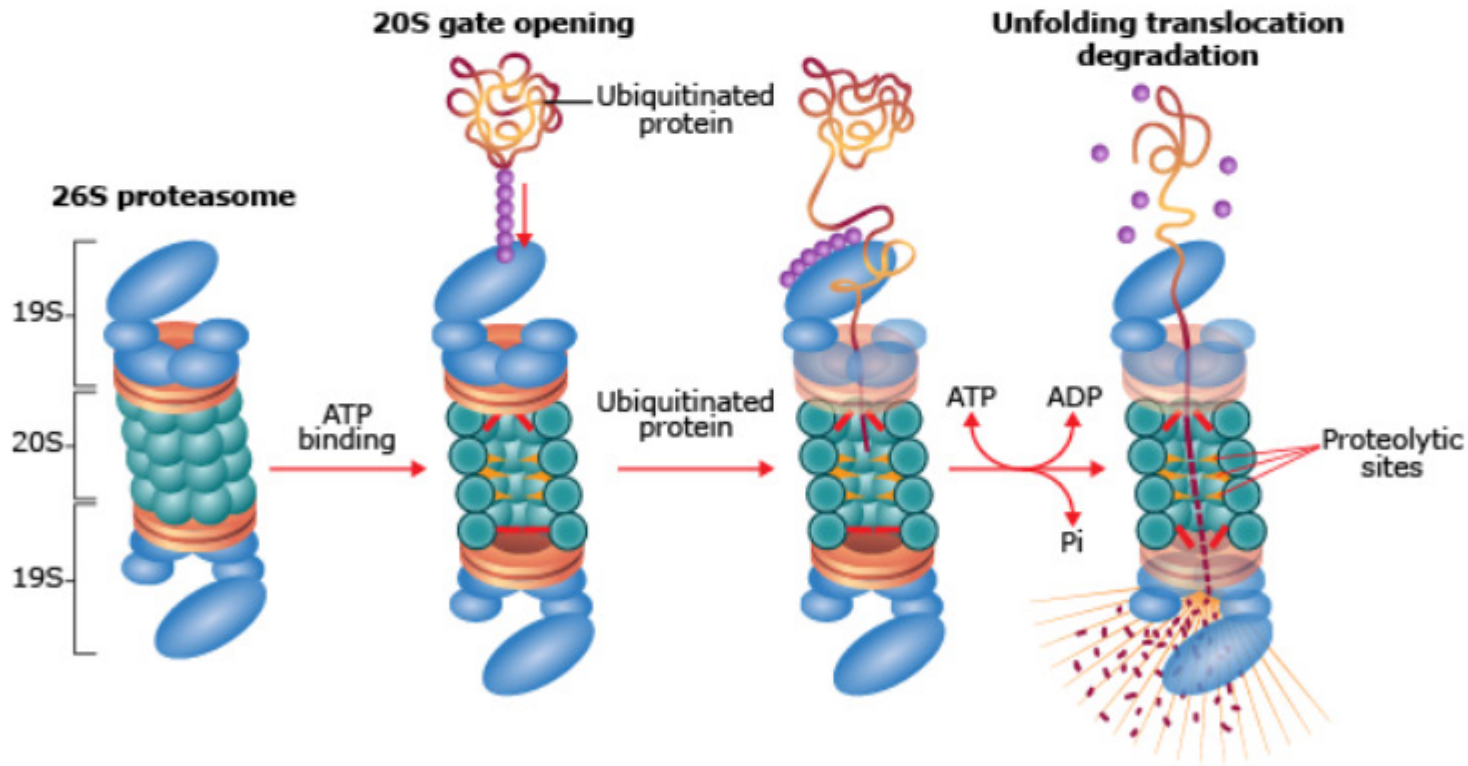


▲ **Figure 4–5.1D** Quarternary Structure

5.2 Chaperones

Folding is usually facilitated by a class of proteins called *chaperones*. There are many different types of chaperone proteins. Several of these were initially named heat shock proteins and were first described in bacteria, where they were found to help proteins renature after exposure to elevated temperatures. These proteins are now referred to more generally as chaperones and known to be present in humans and many other species. The mechanisms by which they act are largely unknown and therefore very unlikely to be tested on the USMLE.

Ultimately, despite the action of chaperones and other factors, if a copy of a protein does not fold correctly, it will be marked for destruction. This typically involves *ubiquitination* and *proteolysis* in structures named *proteasomes*.



▲ **Figure 4–5.2** Proteasome Digestion of Improperly Folded, Polyubiquitinated Protein

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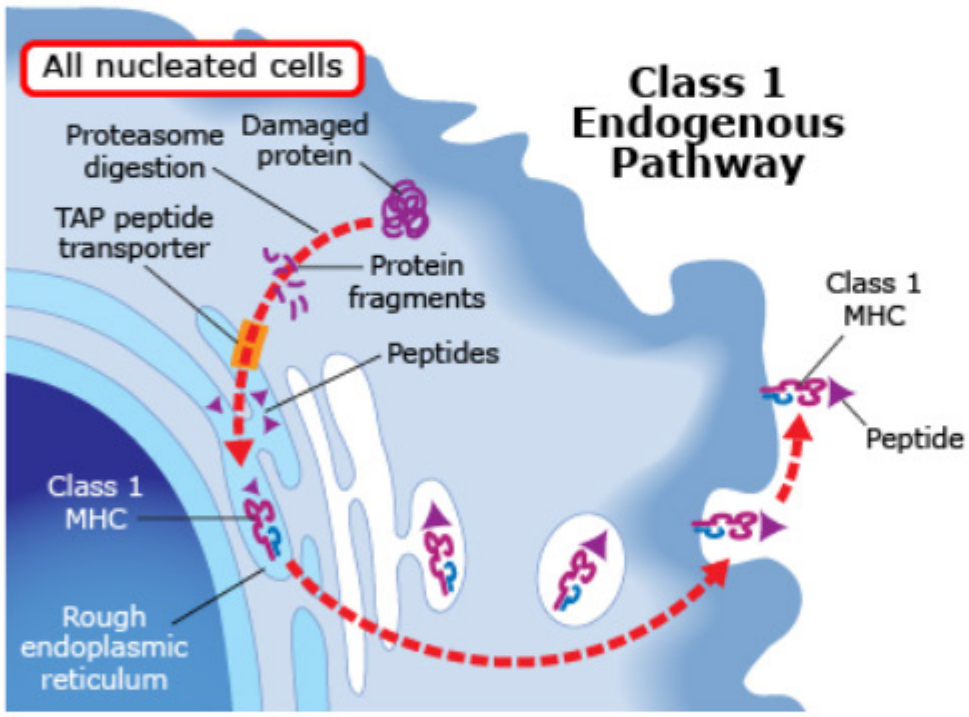
5.3 Proteasomes

Proteasomes are multi-subunit structures found in the cytoplasm and in the nucleus of mammalian cells. They are largely structures that are composed of many proteolytic activities, and digest proteins in the cell that are misfolded and have therefore lost at least part of their native activity. In this role they remove damaged proteins that otherwise might interfere with the function of their active counterparts. The proteasomes distinguish the damaged proteins by the attachment of multiple copies of ubiquitin, a process that is catalyzed by the enzyme *ubiquitin ligase*. These proteins are said to be polyubiquitinated.

5.3.1 Proteasomes and Antigen Presentation to the Cytotoxic T Lymphocyte

When the proteasome has digested a damaged protein, it releases both free amino acids and peptides. These may be further digested and recycled into the cell's general metabolism. Some of the peptides will become associated with class 1 major histocompatibility antigens. These complexes will be presented on the surface of the cell for surveillance by the immune system. This provides a way for cytotoxic T cells to recognize the presence of intracellular pathogens such as viruses, and kill the infected cell, thus preventing further spread of the infection.



▲ **Figure 4–5.3** Proteasome and Antigen Presentation

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5.3.2 Proteasomes and Disease: Cystic Fibrosis

Although the action of proteasomes keeps the cell from accumulating defective proteins, there are well-known instances when they actually contribute to the pathophysiology of a disease. One well-established example is seen in patients with cystic fibrosis who have the ΔF508 mutation. The mutation is an in-frame deletion of three nucleotides from the CFTR (cystic fibrosis transmembrane regulator) gene that deletes a phenylalanine from position 508 in the protein. As a consequence, the CFTR protein fails to fold correctly and activates ubiquitin ligases that mark the protein for degradation in proteasomes. Many experiments have demonstrated that *if* the aberrant CFTR encoded by the ΔF508 mutated gene could be inserted in the cell membrane it would function. (Cystic fibrosis is a highly tested disease on the USMLE).

5.4 Protein Targeting

Almost all eukaryotic proteins are encoded by genes in the nucleus of the cell. When those genes are expressed, translation begins in the cytoplasm. If the protein is destined to function in the cytoplasm, translation will continue on free ribosomes until it terminates and the protein is released into the cytoplasm. An example would be glucokinase in hepatocytes.

5.4.1 Targeting Nuclear Proteins

Proteins that function in cell organelles (e.g., nuclear proteins like RNA or DNA polymerases) will be translated in the cytoplasm. Most of these proteins will have a targeting sequence encoded in their gene that signals the importation of the protein into the appropriate organelle. It is not necessary to memorize the specific amino acid sequences of these different signals.

5.4.2 Targeting Secreted and Integral Membrane Proteins

Proteins that fall into this category include receptors for water-soluble hormones, transporters, ion channels, and many others whose common feature is that they are embedded in the cell membrane. The other group of proteins that follow the same pathway are secreted proteins such as insulin, glucagon, collagen, coagulation factors, and the proteins of the complement cascades. These proteins all begin translation on free cytoplasmic ribosomes. Each of these proteins has an *N-terminal hydrophobic signal sequence* encoded by part of the 5' exon of the gene. As soon as this region is translated into the protein, a signal recognition particle binds to the signal sequence and mediates the attachment of the translation complex to the outer surface of the rough endoplasmic reticulum (RER) where translation continues. The protein is fed through pores in the RER and when translation is terminated it is released into the lumen. While being translated, several co- and posttranslational modifications may be made to the protein.

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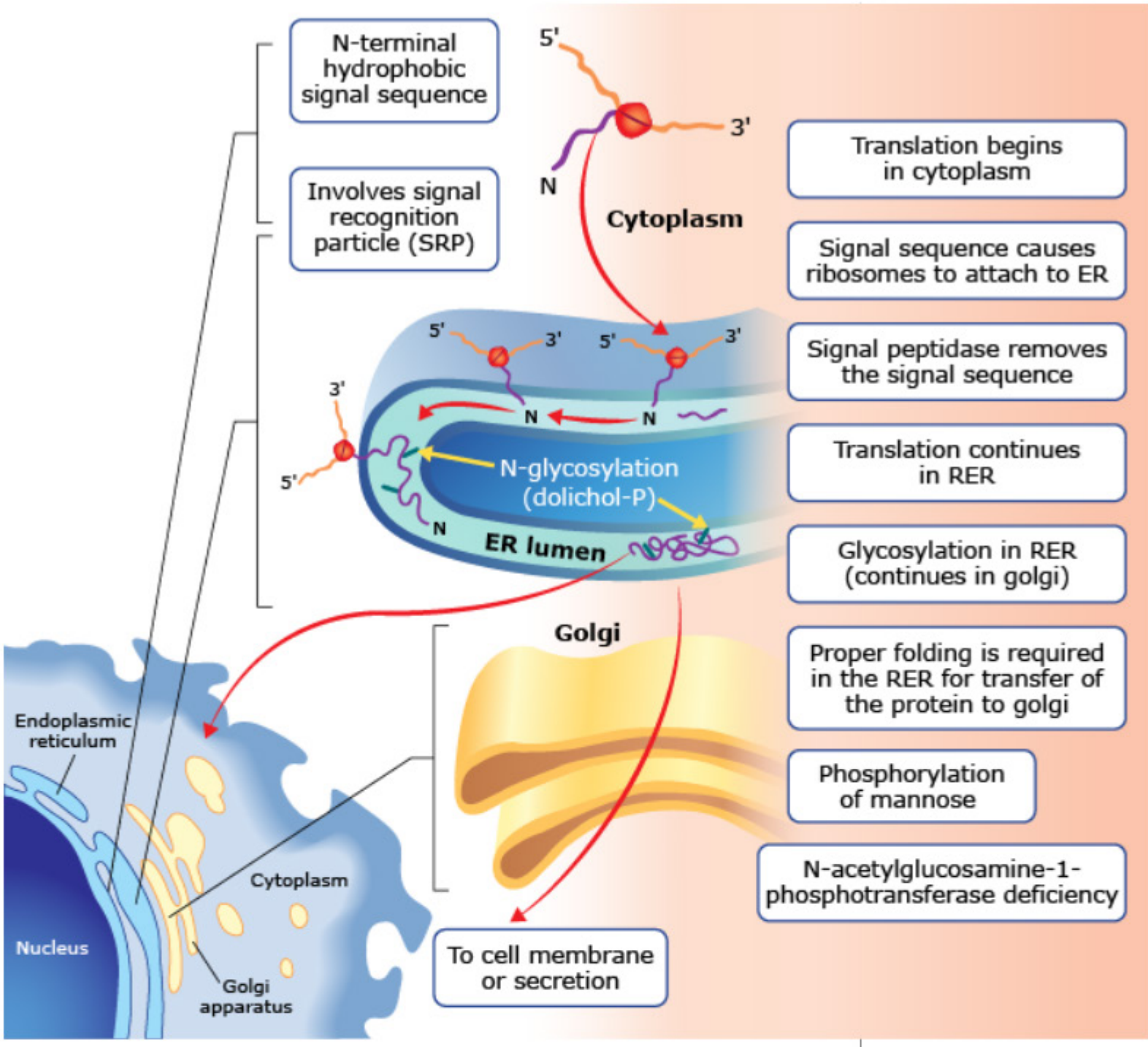

▲ **Figure 4–5.4** Co- and Posttranslational Modification to Secreted, Integral, and Lysosomal Protein

5.5 Co- and Posttranslational Events Occurring in the RER and Golgi

- Signal sequence is removed as the proteins are fed into the lumen of the RER.
- N-linked glycosylation on select asparagine residues (RER).
- O-linked glycosylation of select serine and threonine residues (Golgi).
- Chaperones assist in correct folding of proteins. Failure results in polyubiquitination and digestion in proteasomes (example: ΔF508 mutation in cystic fibrosis).
- If the protein folds *correctly*, it will be moved by vesicular transport to the Golgi, where it will be further modified and sent to the cell membrane by vesicular transport. The vesicles fuse with the cell membrane, releasing their protein cargo outside the cell (example: insulin secretion from cells in the β-islets).

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- For integral membrane proteins, as the vesicles fuse with the cell membrane, the proteins become part of the cell membrane (example: placement of LDL receptor in the hepatocyte membranes).

Important Concept

Coagulation factors II, VII, IX, X, protein C, and Protein S.

5.6 Other Types of Co- or Posttranslational Modifications

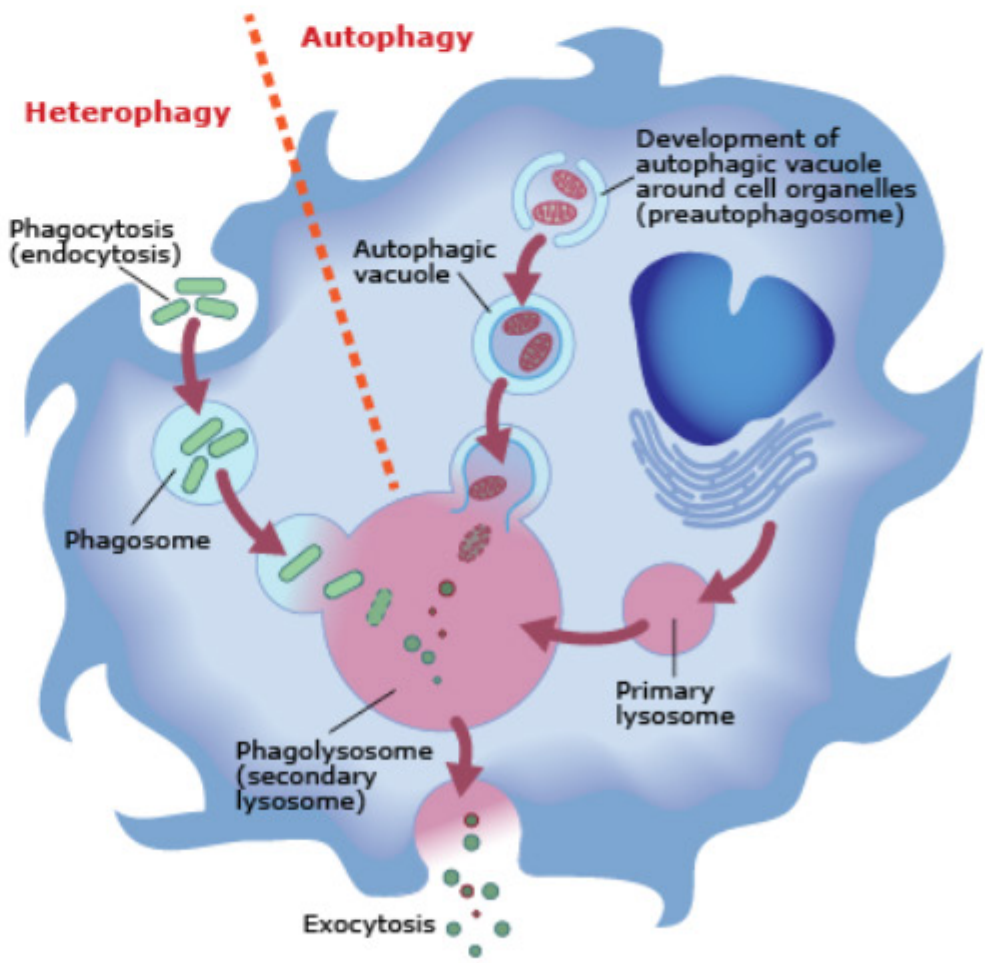
- γ-Carboxylation of glutamic acid in certain Ca^{2+}-binding proteins. The enzyme catalyzing the carboxylation is *γ-glutamyl carboxylase*, which *requires vitamin K*. Warfarin is an antagonist of vitamin K.
- Hydryoxylation of prolyl residues in procollagen α-chains. This modification is catalyzed by *prolyl hydroxylase*, an enzyme that requires *vitamin C*.

5.7 Translation of Lysosomal Enzymes

Similar to secreted and membrane proteins, lysosomal enzymes are also translated to the RER. The genes for these enzymes encode the N-terminal hydrophobic signal sequences, again similar to secreted and integral membrane proteins. After the N-terminal signal sequences is removed, N-linked glycosylation occurs in the RER. Importantly, the newly attached carbohydrate is rich in the sugar mannose. When the lysosomal proteins are transferred to the Golgi, the mannose is phosphorylated in a reaction catalyzed by *N-acetylglucosamine-1-phosphotransferase*. The addition of mannose diverts these enzymes from secretion pathways and targets them to lysosomes, where they await substances brought into the cell by endocytosis or by phagocytosis in "professional" phagocytes such as macrophages.

Lysosomes also slowly sample the cytoplasm by a process of autophagy (self-eating). Together, phagocytosis and autophagy are another strategy for a cell to sample its environment and to digest a wide variety of extracellular and intracellular materials.

As addressed in immunology, the lysosome plays an important role in antigen presentation in the context of class 2 MHC (the exogenous pathway of antigen presentation).



▲ **Figure 4-5.7A** Lysosomes in Phagocytosis and Autophagy

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Clinical Application

Mucolipidosis type II, commonly referred to as *I-cell disease,* is a genetic disease caused by certain mutations in the gene encoding N-acetylglucosamine-1-phosphotransferase.
This disease is frequently tested on the USMLE.
The pathophysiology relates to two key features:

- Absence of key lysosomal enzymes in lysosomes.
- Secretion of those enzymes into the extracellular environment.

Symptoms include:

- Inflammation in tissues
- Joint contractures
- Umbilical hernia
- Macroglossia
- Characteristic facial features (low nasal bridge, anteverted nares, bulging forehead)
- Epicanthic folds
- Growth retardation (growth often stops by 2 to 3 years of age)
- High levels of lysosomal enzymes in blood or serum
- Inclusion bodies within cells (secondary lysosomes filled with indigestible material)

Courtesy of ISMRD

▲ **Figure 4-5.7B** Dysmorphic Appearance of I-Cell Patient

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6 Collagen

6.1 Collagen Function

Collagen, the most abundant protein in the body, is an excellent example of the mechanisms and functions of posttranslational protein processing. It is an extracellular structural protein that functions to organize and provide structure to connective tissues. It provides tensile strength to ligaments, tendons, and bones.

6.2 Collagen Structure

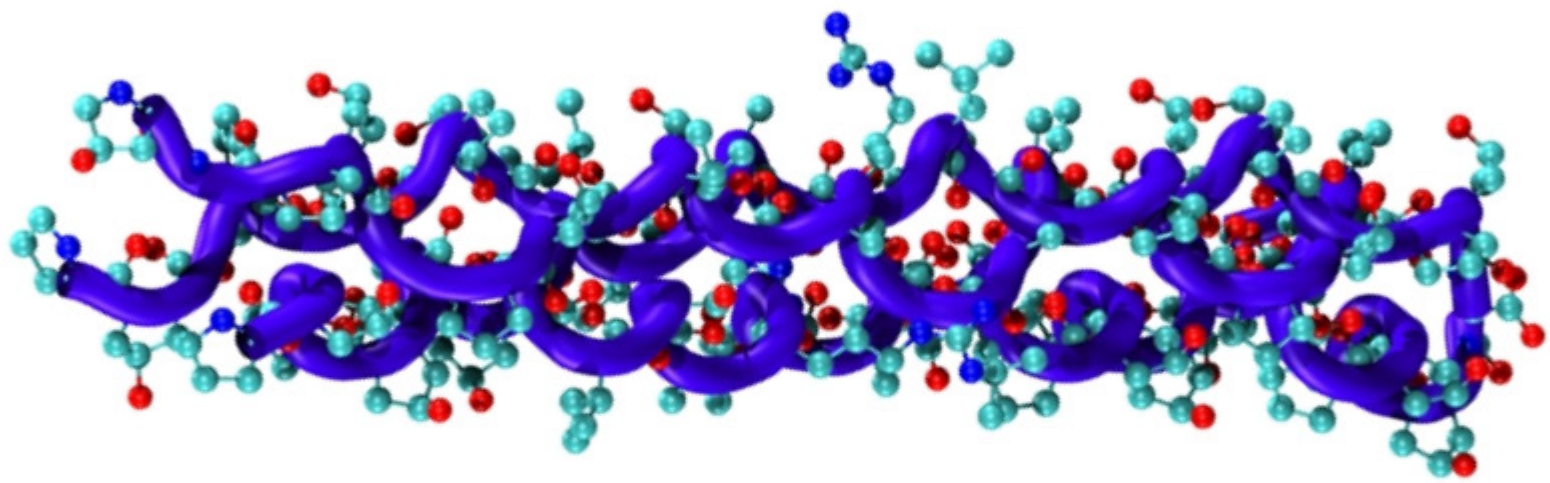
The structure and function of collagen arises from its distinctive amino acid composition. Every third amino acid in collagen is a glycine. About 20% of the amino acids are proline, many of which are modified by hydroxylation (forming hydroxyproline) after translation. Some lysine amino acids are hydroxylated; others are deaminated.

Important Concept

Glycine is the most abundant amino acid in collagen.

Hydroxylation adds an -OH to the amino acid.

Deamination removes the amine group from a molecule.



Kallista Images/Custom Medical Stock Photo

▲ **Figure 4-6.2A** Collagen Structure

The structure of collagen is like a rope, with multiple fibers wound around each other to give it strength. The basic fiber of collagen is the collagen peptide itself, which consists of a long left-handed (counter-clockwise) alpha helix. This conformation is due to the glycine amino acids at every third amino acid. Three collagen peptides wind around one another in a right-handed (clockwise) triple helix. This structure is due to interaction of hydroxyproline and hydroxylysine amino acids.

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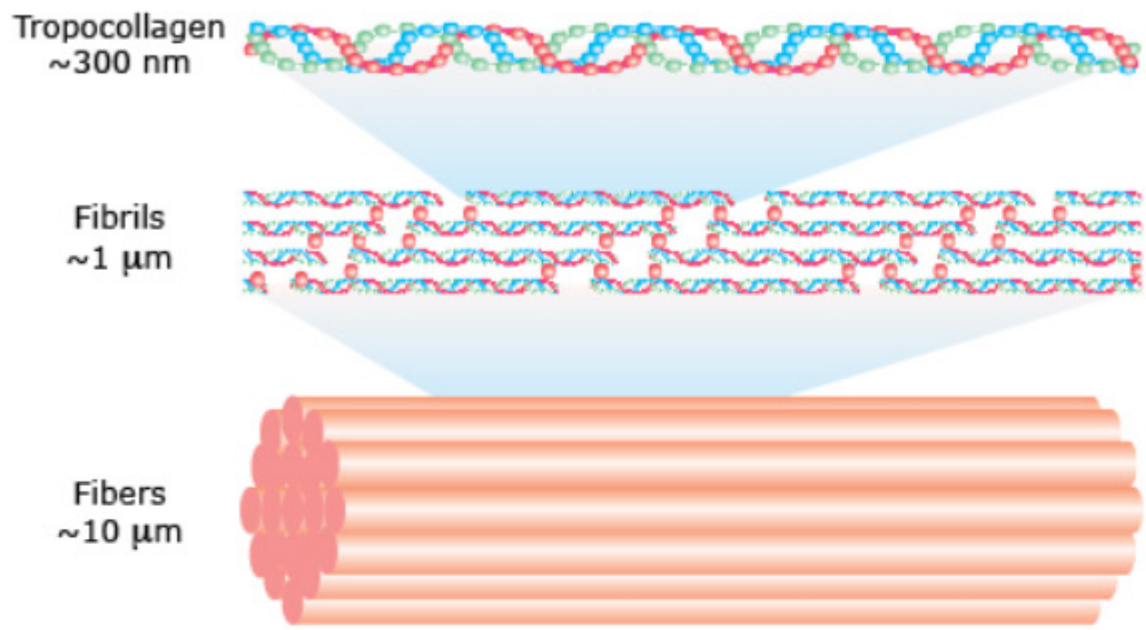
These triple helices are secreted into the extracellular space, where they associate with each other to form collagen fibrils. These are cross-linked to each other by covalent bonds between deaminated lysines. Fibrils then associate with each other to form collagen fibers.



▲ **Figure 4-6.2B** Collagen Structure From Amino Acid to Collagen Fibers

Collagen provides an excellent example of the layers of protein structure:

- **Primary structure:** The regular arrangement of glycine residues and high content of proline.
- **Secondary structure:** The α-helical conformation of individual collagen peptides.
- **Quaternary structure:** The interactions between collagen peptides and fibrils through hydrophobic and polar interactions and covalent bonds.

6.3 Sites and Mechanisms of Collagen Synthesis

Collagen peptides (preprocollagens) are synthesized in the rough endoplasmic reticulum (RER). Formation of the triple helix (procollagen) also occurs in the RER. Hydroxylation occurs in the RER and is catalyzed by prolyl hydroxylase and lysyl hydroxylase. Fibril formation, lysine deamination (catalyzed by lysyl oxidase), and cross-linking occur in the extracellular space.

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6.4 Types of Collagen

Multiple collagen genes encode peptides with sequence variations. These associate in various combinations to form distinct types of collagen with specific functions and tissue distributions:

- **Type I**—skin, bones, tendons, cornea
- **Type II**—cartilage
- **Type III**—blood vessels, loose connective tissue (e.g., dermis)
- **Type IV**—epithelial basement membranes

6.5 Collagen Disorders

6.5.1 Osteogenesis Imperfecta (OI)

OI is caused by one of many described mutations in various collagen-encoding genes:

- These include mutations causing decreased production of collagen. These mutations are often autosomal or X-linked recessive.
- Others are point mutations, often in glycine codons, that disrupt the normal structure of collagen. These mutations are autosomal dominant because altered structure of one peptide can disrupt the structure of the whole molecule, even if other normal peptides are present.

There are four different types of OI:

- *Type I* is autosomal dominant and exhibits skeletal fragility with frequent fractures, dental deformities, hearing impairment due to deformities in the middle ear ossicles, lax joints due to weak tendons, and blue sclera due to thin connective tissue.
- *Type II* is mostly autosomal dominant and less commonly autosomal recessive. It is the most severe form of OI—often perinatally lethal—with severe skeletal fragility and deformity.
- *Type III* is mostly autosomal dominant and less commonly autosomal recessive with features similar to type I, including growth retardation and spinal kyphoscoliosis (defects in the curvature of the spine), but less severe.
- *Type IV* is autosomal dominant. It is the least severe with variable or absent skeletal fragility, dental deformities, and short stature.

6.5.2 Ehlers-Danlos Syndrome

Ehlers-Danlos syndrome is similar to OI; it's also caused by mutations in type I or type III collagen. It is a very heterogeneous disease, with variable symptoms, including hyperextensible joints and hyperelastic skin, vessel instability with frequent skin hemorrhage, and increased risk of dissecting aortic aneurysm or colon rupture.

6.5.3 Scurvy

This is an acquired collagen defect due to dietary deficiency of vitamin C (ascorbic acid). Vitamin C is a required cofactor for prolyl and lysyl hydroxylases. Deficiency causes decreased proline and lysine hydroxylation with resulting defects in collagen structure. The clinical consequences include thin skin with defective wound healing, easy bruising, bleeding gums, and abnormal bone growth.

Activities in the RER:

- Formation of preprocollagen
- Formation of triple helix
- Hydroxylation

Activities in the extra-cellular space:

- Fibril formation
- Lysine deamination
- Cross-linking

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1 Mutations

1.1 Types of Mutations

A mutation is defined as a permanent change in DNA sequence. There are three types of mutations:

- Substitution mutation
- Insertion mutation
- Deletion mutation

1.1.1 Substitution Mutation

These mutations, also known as point mutations, are substitutions of one nucleotide for another nucleotide at the same site. Substitution mutations do not change the total number of nucleotides in a DNA sequence. There are two types of substitutions:

- **Transition:** A transition involves substitution of a like nucleotide, for example, a purine for another purine (e.g., A → G) or a pyrimidine for another pyrimidine (e.g., T or U → C).
- **Transversion:** A transversion involves substitution of an unlike nucleotide, for example, a purine for a pyrimidine (e.g., G → T or U) or a pyrimidine for a purine (e.g., C → A).

▲ **Figure 5–1.1A** Transition and Transversion

There are four potential consequences of nucleotide substitution mutations:

- **Silent Mutation:** Silent mutations do not change the amino acid encoded by the mutated codon. These usually occur at the third position of the codon, where there is the greatest redundancy in the codon table; however, they can on occasion occur at the first position as well. For example, GGC encodes glycine. If the third nucleotide is mutated to G (a transversion), the codon will now be GGG, which also encodes glycine.

```
5' –AUG CAU UGU GGC AGA CCA– 3'
    Met His Cys Gly Arg Pro

5' –AUG CAU UGC GGG AGA CCA– 3'
    Met His Cys Gly Arg Pro
```

▲ **Figure 5–1.1B** Silent Mutations

USMLE® Key Concepts

For Step 1, you must be able to:

- Describe the three kinds of DNA mutations and their consequences.
- Explain the causes of DNA mutation and mechanisms of repair.

Important Concept

Transition changes a purine for a different purine, or a pyrimidine for another pyrimidine.

Transversion changes a purine for a pyrimidine, or a pyrimidine for a purine.

Important Concept

Four outcomes of substitution mutations:

- Silent
- Missense
- Nonsense
- Splice site

Memory Aid

Silent mutations still code for the same amino acid. They are *silent* because *no one complains.*

Silent mutations do not alter phenotype.

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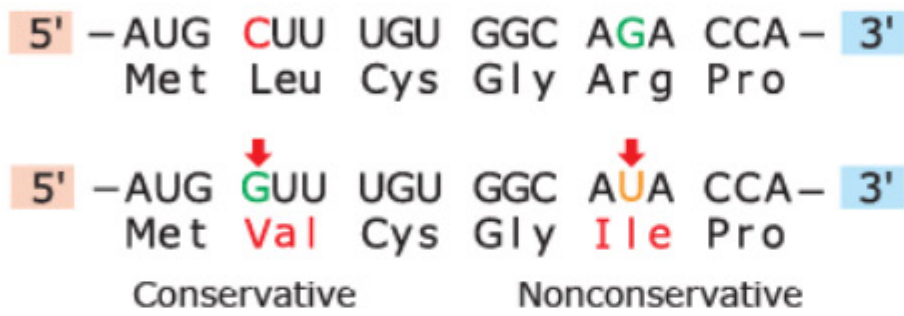
- **Missense Mutation:** Missense mutations change the codon from that of one amino acid to another. These most commonly occur at the first and second position in the codon, although they occasionally occur at the third position as well. The functional consequence of a missense mutation depends, in part, on the specific amino acid change.
  - **Conservative Mutation:** Converts the encoded amino acid to another with similar properties. For example, CUU encodes leucine, an aliphatic amino acid. If the first nucleotide is mutated to G (a transversion), the codon will now be GUU, which encodes valine. Valine is also aliphatic, so the consequences for protein function are predicted to be minor.
  - **Nonconservative Mutation:** Converts the encoded amino acid to another with different properties. For example, AGA encodes arginine, a positively charged amino acid. If the second nucleotide is mutated to U (a transversion), the codon will now be AUA, which encodes isoleucine. Since isoleucine is aliphatic, not charged, this mutation is predicted to have a more significant effect on protein function.



▲ **Figure 5–1.1C** Conservative and Nonconservative Mutation

- **Nonsense Mutation:** Nonsense mutations change an amino acid-encoding codon to a stop codon (TAG, TAA, or TGA in DNA; UAG, UAA, or UGA in the corresponding mRNA). For example, UGU encodes cysteine. If the third nucleotide is mutated to A (a transversion), the codon will now be UGA, a stop codon. Nonsense mutations cause early termination of translation, resulting in truncated proteins. This usually has functional consequences, because it significantly alters the protein structure.

▲ **Figure 5–1.1D** Nonsense Mutation

- **Splice Site Mutation:** Processing of hnRNA to form mRNA requires the removal of introns (see chapter 3, 6.4). The spliceosome recognizes specific sequences (GU and AG) at the beginning and end of each intron. Thus, mutation of these sites in the corresponding DNA could block intron splicing and prevent the formation of the proper mRNA. This could significantly reduce gene expression.

Important Concept

Conservative mutations result in change to an amino acid with similar chemical properties.

Clinical Application

Sickle cell disease is an example of nonconservative missense mutation. A is substituted for T at the 17th nucleotide of the gene for the beta chain of hemoglobin, causing glutamic acid to be replaced by valine.

Looking Back

Stop codons:

- UGA—**U** **G**o **A**way
- UAG—**U** **A**re **G**one
- UAA—**U** **A**re **A**way

Clinical Application

Splice site mutations in the β-globin gene are common causes of β-thalassemia.

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1.1.2 Insertion Mutation

These mutations are insertions of one or more nucleotides into a sequence. This has three potential consequences:

- If the insertion is a multiple of three nucleotides (3, 6, 9, etc.), this simply adds amino acids to the protein. For example, here is an RNA sequence:

```
5' –AUG CAU GGG UGU CGA CCA– 3'
     Met His Gly Cys Arg Pro
```

The following polypeptide is one amino acid longer, but there are no other changes.

```
5' –AUG CAU GGG UGU GGC CGA CCA– 3'
     Met His Gly Cys Gly Arg Pro
```

- If the insertion is not a multiple of three but some other number of nucleotides, there is a shift in the reading frame, such that there is a change in the coded amino acids in the 3' direction immediately following the mutation. For example, here is an RNA sequence:

```
5' –AUG CAU GGG UGU GGG AGA CCA– 3'
     Met His Gly Cys Gly Arg Pro

5' –AUG CAA UGG GUG UGG GAG ACC A– 3'
     Met Gln Trp Val Trp Glu Thr
```

▲ **Figure 5–1.1E** Frameshift Mutation

Note that the reading frame shifts, such that the encoded amino acid sequence downstream of the mutation is completely altered. This type of insertion mutation (known as a frameshift mutation) generally has a significant effect on protein function.

- Frameshifts very often create new stop codons. For example, here is an RNA sequence:

```
5' –AUG CAU UGU GAC AGA CCA– 3'
     Met His Cys Asp Arg Pro

5' –AUG CAA UUG UGA CAG ACC A– 3'
     Met Gln Leu Stop
```

▲ **Figure 5–1.1F** Frameshift Mutation: Stop Codon

Note that the reading frame shifts, creating a stop codon two codons down from the mutation. This would result in a truncated protein. This generally has a significant effect on protein function.

Clinical Application

Both fragile X syndrome and Huntington disease are caused by trinucleotide repeat insertions. Fragile X syndrome repeats CGG, and Huntington repeats CAG.

Some forms of adult onset muscular dystrophy contain tri- or tetranucleotide repeat insertions of CTG and CCTG.

Important Concept

Both insertion and deletion mutations can cause frameshift mutations.

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1.1.3 Deletion Mutation

Deletion mutations involve deletions of one or more nucleotides from a sequence, shortening the chromosome. Deletions have the same potential consequences as insertion mutations:

- A deletion that is some multiple of three nucleotides will shorten the protein by removal of one or more amino acids, but will not alter the reading frame.
- A deletion of any other number of nucleotides will shift the frame, changing the encoded amino acid sequence downstream of the mutation.

▲ **Figure 5–1.1G** Deletion of One Nucleotide

- Frameshift mutations often lead to early stop codons, resulting in protein truncation.

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2 DNA Repair

2.1 Causes of Mutations

Mutations can be caused by mistakes in DNA replication, as discussed previously. They can also be caused by environmental damage that alters the structure of nucleotides. Replication of damaged nucleotides often changes the nucleotide, leading to a substitution mutation, insertion mutation, or deletion mutation. Common sources of environmental damage include the following:

- Heat can cause loss of bases or deamidation, converting one nucleotide to another.
- Ultraviolet light can cause covalent bonds between adjacent pyrimidines, called *pyrimidine dimers*.
- X-rays can cause loss of bases or can break bonds between nucleotides and the DNA backbone.
- Many chemicals can modify nucleotides by alkylating, methylating, or cross-linking DNA.
- Intercalating agents.

2.2 Mechanisms of DNA Repair

2.2.1 Mismatch Repair

The mismatch-repair complex recognizes mismatched base pairs, such as those that occur with replication errors, and repairs them in four steps:

- An endonuclease breaks one of the phosphodiester bonds 5' of the mismatched nucleotide.

▲ **Figure 5–2.2A** Nick in DNA Strand

- A 5' → 3' exonuclease removes the nucleotides around and including the mismatched nucleotide.

▲ **Figure 5–2.2B** Segment of DNA Removed

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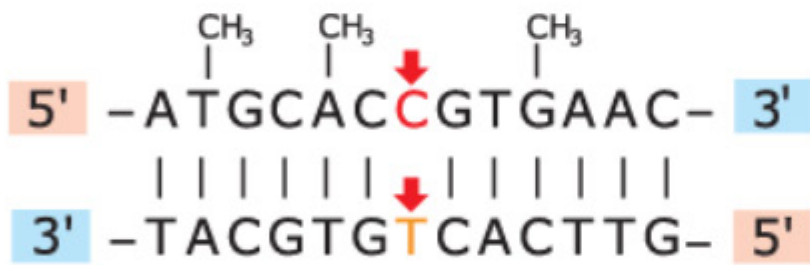
- DNA polymerase (β) fills in the gaps using the opposite DNA strand as a template.

▲ **Figure 5–2.2C** DNA Filled In

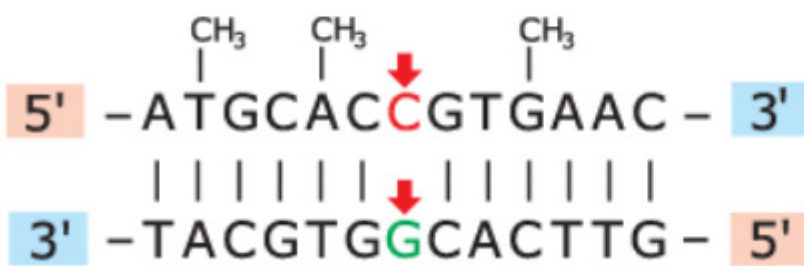
- DNA ligase creates a phosphodiester bond between the last newly synthesized nucleotide and its 3' neighbor to complete the repair.

How does the DNA repair complex know which mismatched nucleotide is the correct one and which is the mismatched one? Prior to replication, the parental DNA is methylated; that is, methyl groups are added to some nucleotides. Typically the mismatched nucleotide will be in the daughter strand, which is recognized by the DNA repair complex as the non-methylated strand.

▲ **Figure 5–2.2D** Problematic DNA Strand



▲ **Figure 5–2.2E** DNA Strand Is Methylated



▲ **Figure 5–2.2F** Repaired DNA Strand

Important Concept

Enzyme steps in mismatch repair:

1. Endonuclease
2. Exonuclease
3. DNA polymerase (β or ε)
4. Ligase

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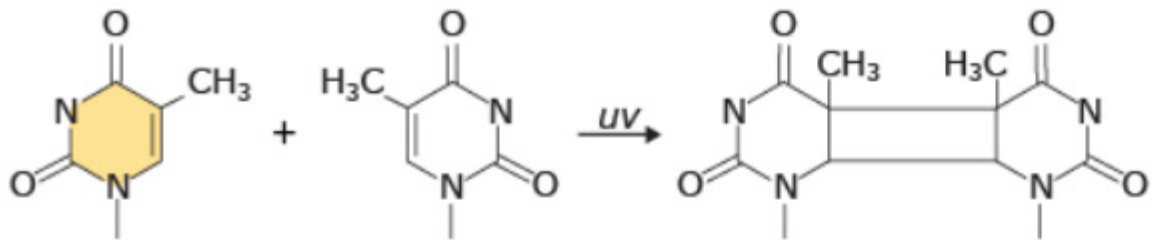
2.2.2 Pyrimidine Dimer Repair



▲ **Figure 5–2.2G** Thymine Dimer

Remember that pyrimidine dimers are created by exposure to UV radiation. An endonuclease recognizes the dimer and cleaves phosphodiester bonds on both the 5' and 3' ends of the damaged segment.

```
5'  -ATAGCT=TAGAAC-  3'
     | | | | | |   | | | | | |
3'  -TATCGA-ATCTTG-  5'
```

▲ **Figure 5–2.2H** Dimer

```
5'  -ATAG        GAAC -  3'
     | | | |     | | | |
3'  -TATCGA-ATCTTG -  5'
```

▲ **Figure 5–2.2I** Strand Cleavage

The resulting gap is then filled in by the mismatch-repair complex described above.

```
5'  -ATAGCT-TAGAAC-  3'
     | | | | | |   | | | | | |
3'  -TATCGA-ATCTTG-  5'
```

▲ **Figure 5–2.2J** Repaired DNA Strand

Important Concept

Only thymine and cytosine can form pyrimidine dimers.

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2.2.3 Base Excision Repair

Base alteration can occur by many mechanisms, including spontaneous deamidation or chemical exposure, such as exposure to deaminating or alkylating agents.

▲ **Figure 5–2.2K** C Is Changed to U

These damaged bases are recognized by specific glycosylases that cleave the base from the deoxyribose backbone, leaving an apurinic or apyrimidinic site.

▲ **Figure 5–2.2L** U Base Is Cleaved

The 5' phosphodiester bond is then broken by an endonuclease and the deoxyribose phosphate removed by deoxyribose phosphate lyase.

▲ **Figure 5–2.2M** Deoxyribose Phosphate Is Removed

The resulting gap is filled in by DNA polymerase and ligase.

▲ **Figure 5–2.2N** Base Filled In

Important Concept

Enzyme steps in base excision repair are as follows:

1. DNA glycosylase
2. DNA endonuclease
3. Deoxyribose phosphate lyase
4. DNA polymerase
5. DNA ligase

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2.3 DNA Repair Defects

2.3.1 Fanconi Anemia

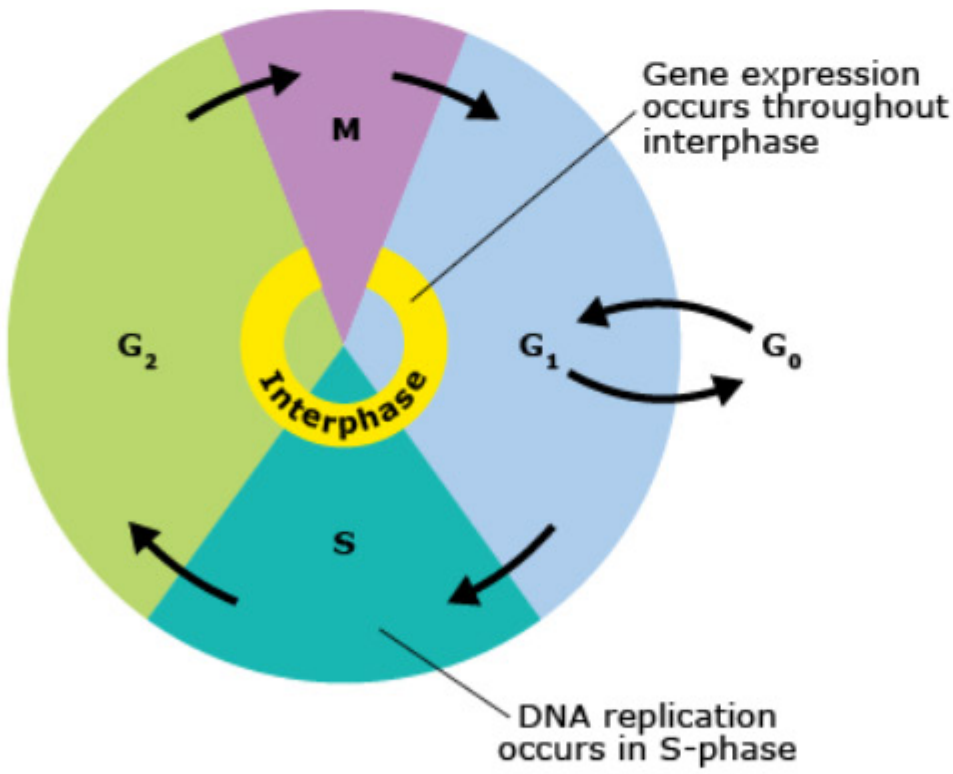
These patients exhibit sensitivity to damage caused by DNA cross-linking agents. They also exhibit congenital skeletal malformations and progressive aplastic anemia. They are susceptible to developing acute myeloid leukemia (AML).

2.3.2 Ataxia Telangiectasia

This disease is caused by a defect in the ATM gene, which codes for a protein critical to the cell response to a several forms of stress, including double-strand breaks in DNA. As a consequence, patients exhibit sensitivity to damage caused by x-rays. They have numerous oculocutaneous telangiectasias (on eyes and skin). They also have progressive cerebellar ataxia and are relatively immunodeficient. They are particularly susceptible to lymphoid malignancies.

2.4 Cell Cycle Control

DNA repair needs to occur prior to DNA replication; if not, mutations will be replicated and then propagated into all the daughter cells. There are checkpoints in the cell cycle at which the cell cycle can be halted in the presence of DNA damage; the primary checkpoint is between G_1 and S phases. The retinoblastoma protein (Rb) and the p53 transcription factor (p53) are two of the proteins involved in the regulation of this transition; they are activated in the presence of DNA damage and block the transition.



▲ **Figure 5–2.4** Rb and p53 in the Cell Cycle

Important Concept

Common mismatch repair defects include the following diseases (defect in parentheses):

- Ataxia telangiectasia (ATM)
- Hereditary nonpolyposis colorectal cancer (MSH, MLH)
- Xeroderma pigmentosum (UVR, ABC)
- Breast cancer (BRCA1/2)
- Werner syndrome (Helicase)

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2.5 Li-Fraumeni Cancer Syndrome

Mutations in p53 lead to an inherited cancer predisposition called Li-Fraumeni cancer syndrome. These mutations also allow cell cycle progression (through the G_1/S checkpoint) in the presence of DNA damage, causing mutations that lead to cancer. Patients with this disease develop common cancers, including breast cancer and soft tissue sarcomas, at a greater rate and a younger age.

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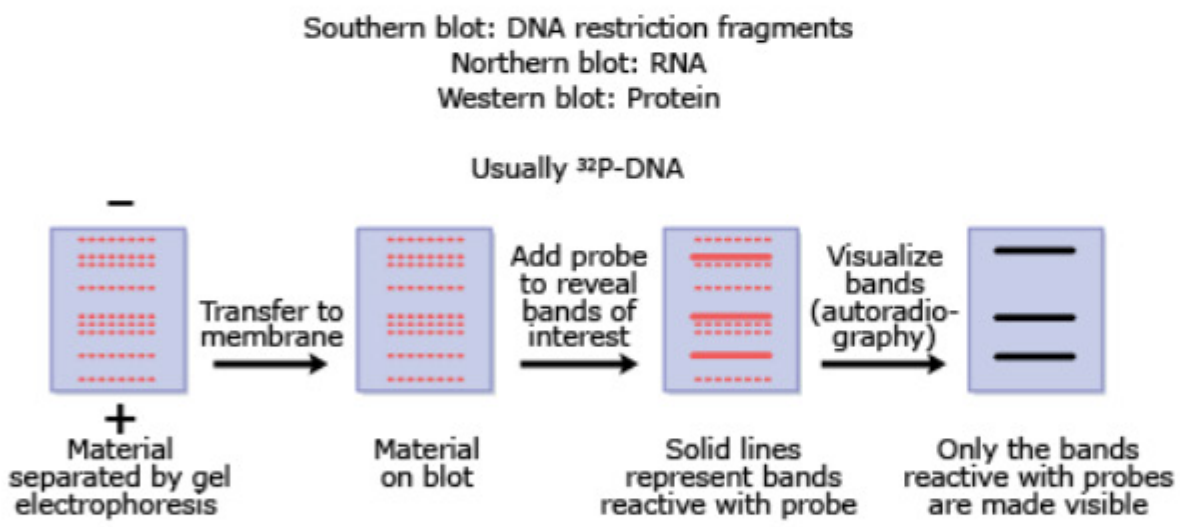
1 Techniques of Genetic Analysis

Molecular biological techniques of genetic analysis are increasingly important in medical diagnosis. Blotting techniques are used to test for genetic diseases and to identify microbial antigens and the antibodies formed against them.

1.1 Blotting Techniques

Blotting techniques have been developed to identify DNA, RNA, and proteins from complex mixtures of those substances. The Southern blot identifies DNA fragments, the Northern blot identifies RNA fragments, and the Western blot identifies proteins. In each of these cases, the material to be analyzed is separated by gel electrophoresis and then fragments in the gel are transferred to a membrane. The membrane is incubated with a radioactively labeled probe that will specifically bind to the material being identified, and the bands of probe binding are visualized by autoradiography.



▲ **Figure 6–1.1A** Blotting Techniques

1.1.1 Probes

The probes used for band identification of a blot are typically labeled with ^{32}P or ^{125}I. In the case of the Southern blot, the probe is labeled complementary DNA. Its purpose is to determine which restriction fragments are associated with a particular gene. In the case of Northern blots, the probe is labeled complementary DNA, and the purpose is to identify specific mRNA molecules and learn about gene expression. In the Western blot, the probe is a labeled antibody, and the purpose of the assay is to detect protein antigens or antibodies.

USMLE® Key Concepts

For Step 1, you must be able to:

- Explain the application of DNA-detecting methodologies in the identification of polymorphism.
- Describe the applications and interpretation of data from Northern, Southern, and Western blots.
- Identify the uses of PCR and RT-PCR in genetic and microbial diagnosis.
- Explain the techniques of recombinant DNA technology, gene therapy, and transgenic and knockout mice.

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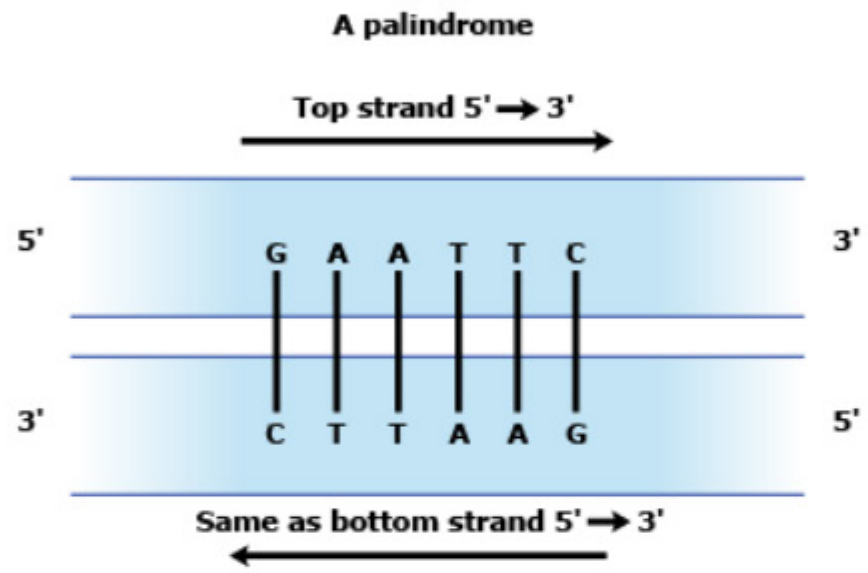
1.1.2 Producing Restriction Fragments of DNA

To analyze DNA, chromosomes, which may be hundreds of millions of base pairs in length, must be broken into manageably-sized pieces. This can be done using specific enzymes called *restriction endonucleases*. These are produced by bacteria as a defense mechanism from bacterial viruses (phages). Each restriction endonuclease cuts DNA at a specific sequence called a recognition sequence. These sequences are usually *palindromes,* meaning that the two strands read exactly the same in the 5' → 3' direction.

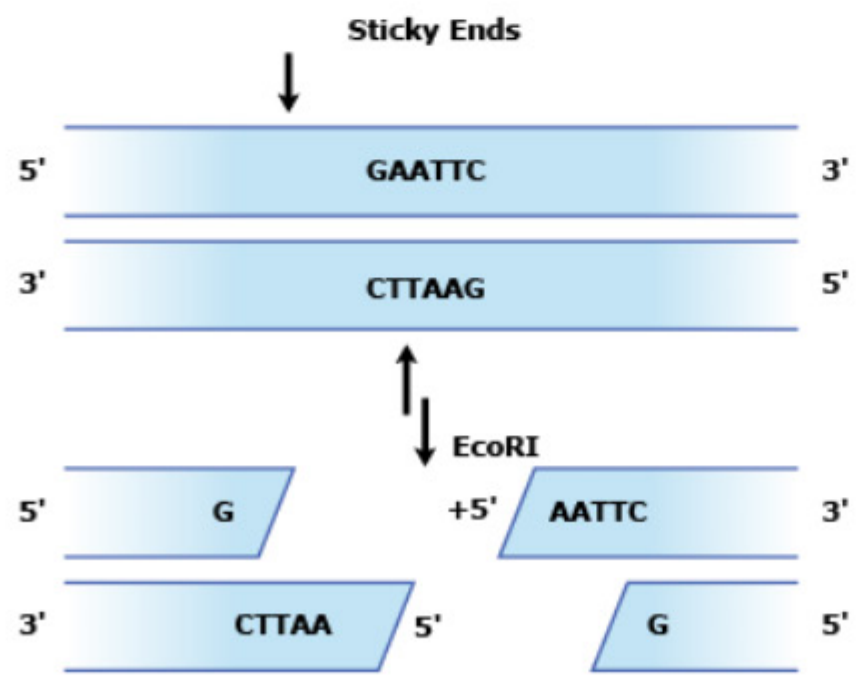
In figure 6–1.1B, the restriction endonuclease EcoRV cuts the sequence 5'-GAATTC-3'. Notice that the antiparallel and complementary sequence to this is also 5'-GAATTC-3'.



This is the DNA sequence recognized by the restriction endonuclease EcoRI.

▲ **Figure 6–1.1B** EcoR1 Recognition Sequence

Restriction endonucleases can cleave DNA at the same site on both strands, leaving a blunt end, with no overhangs, or at different sites, leaving an unpaired overhang, often called "sticky" ends, because they can easily pair with other fragments containing a complementary overhang.



▲ **Figure 6–1.1C** Action of Restriction Endonucleases

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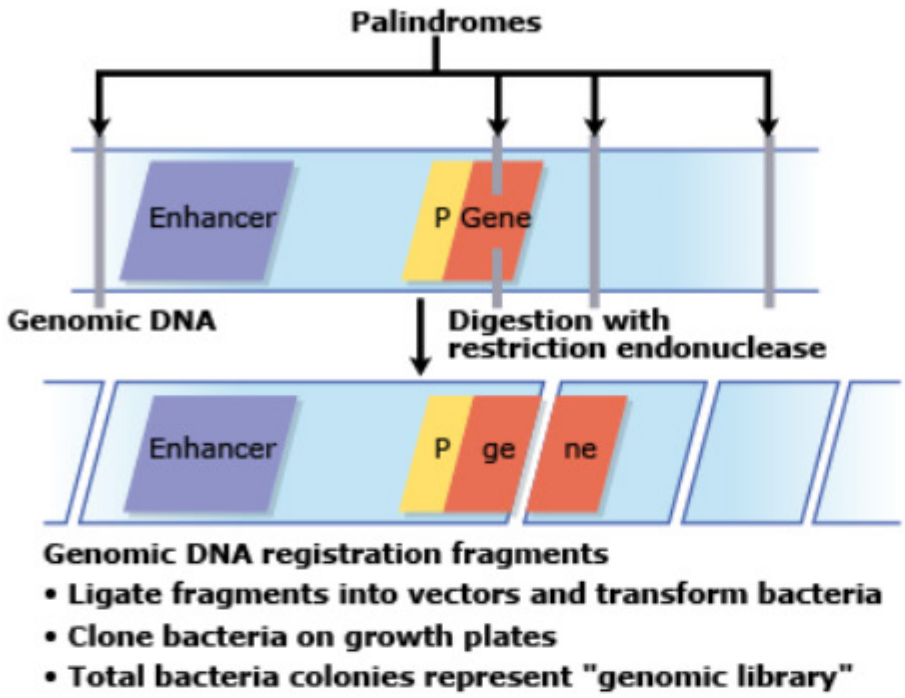
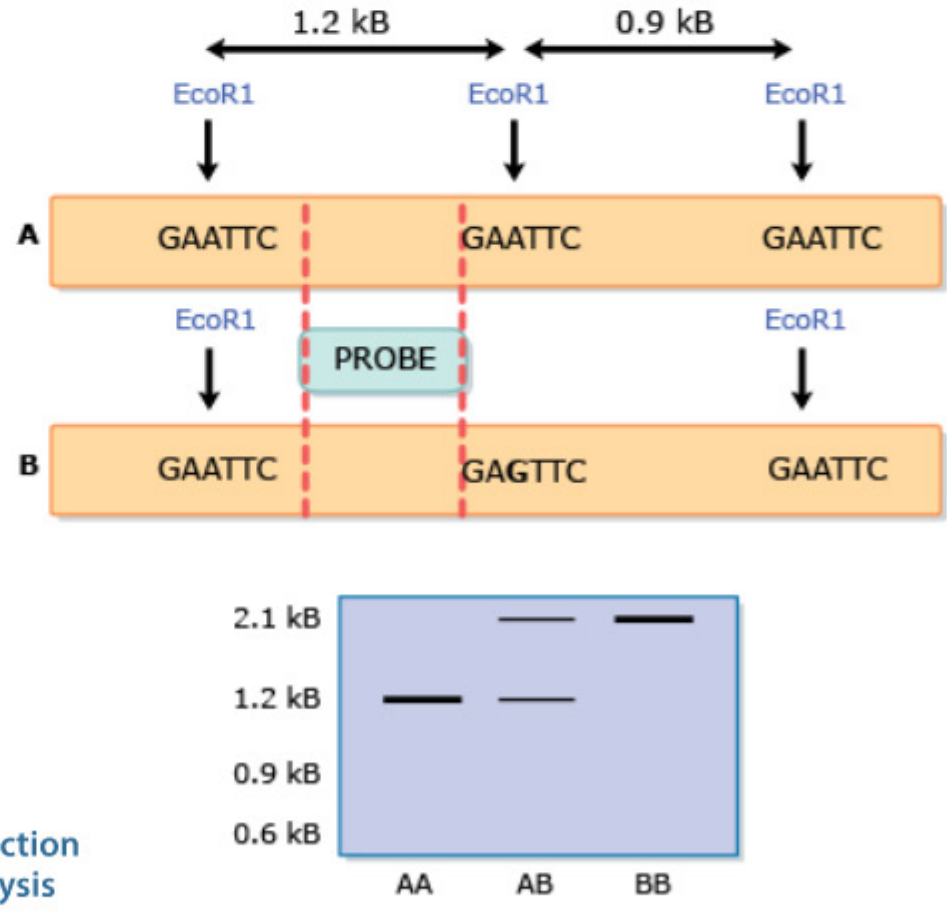
Once the target DNA to be cloned is treated with restriction endonucleases to create restriction fragments, each fragment must be inserted into a vector.



▲ **Figure 6–1.1D** DNA Digestion With a Restriction Endonuclease

1.1.3 Southern Blots and Restriction Fragment Length Polymorphisms

Restriction fragment length polymorphisms (RFLPs) and genetic testing polymorphisms include differences in target sequences for restriction endonucleases, such that some individuals carry certain sequences and others do not. As a consequence, the size of DNA fragments that result from different restriction endonucleases differs among individuals. These differences, known as RFLPs, can be used to identify a sample from a particular individual or to determine the genotype of an individual at a particular DNA locus.



▶ **Figure 6–1.1E** Southern Blot: Restriction Fragment Length Polymorphism Analysis

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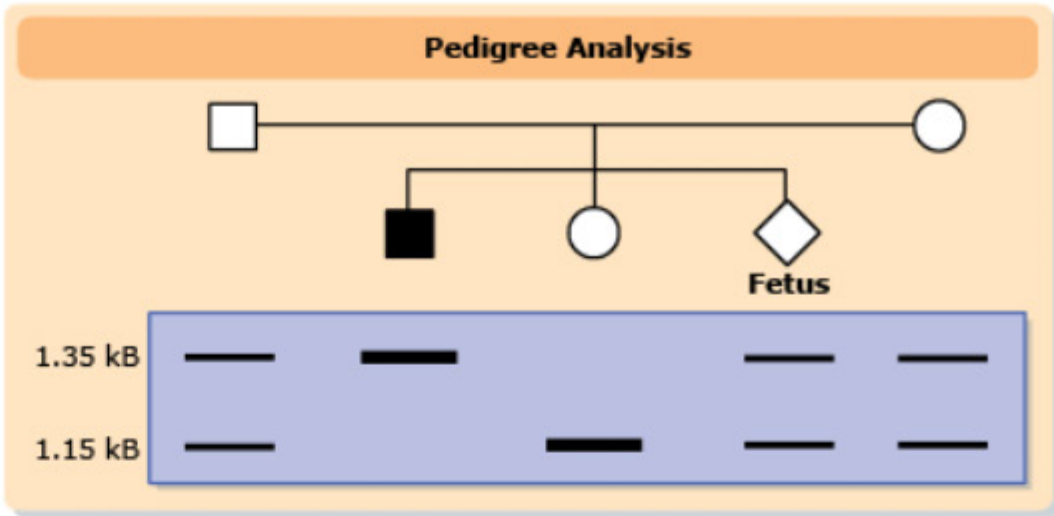
1.1.4 Genetic Testing Using RFLPs

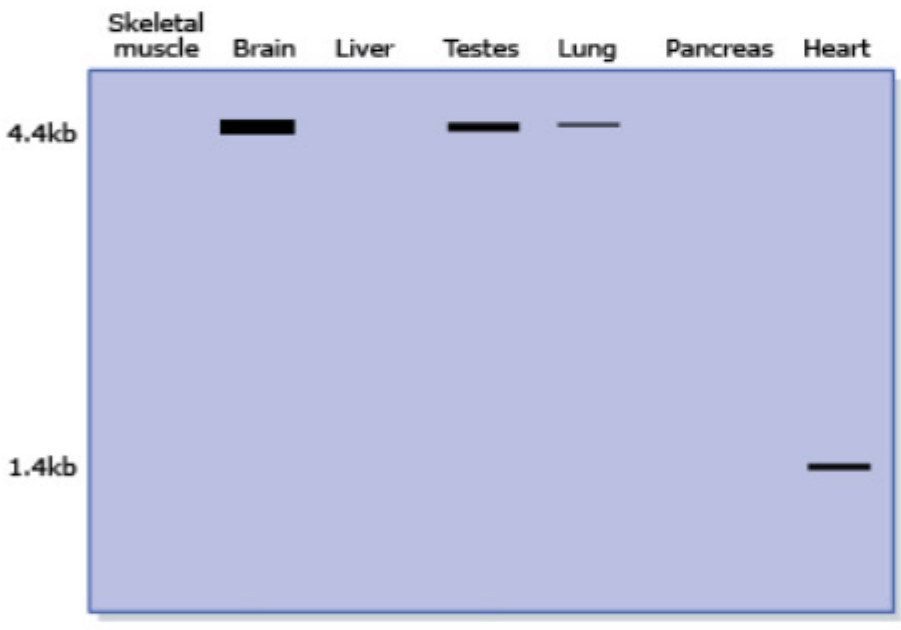
If disease-causing mutations alter the existence of restriction endonuclease sites, cleavage of disease-gene-bearing versus normal-gene-bearing chromosomes can produce RFLPs that can be identified as markers of genotype. When a family wishes to know if a fetus will be affected with disease, these RFLPs can be diagnostic. In the case represented in figure 6-1.1F, notice that the fetus is a heterozygous carrier.



▲ **Figure 6–1.1F** RFLP Analysis in Sickle Cell Disease

1.1.5 Northern Blots

Northern blots are used to analyze RNA and measure gene expression. Notice in figure 6-1.1G that the gene is heavily expressed in brain and testes, and to a lesser extent in lung and heart. The different lengths of the transcripts observed could be due to alternative RNA splicing.



▲ **Figure 6–1.1G** Northern Blot: Determining Gene Expression

1.1.6 Microarrays

Probes for many different mRNAs can be embedded in gel or on microchips to simultaneously measure patterns of gene expression in a tissue. This can be particularly useful in analysis of tumor cell attributes and development of treatment protocols.

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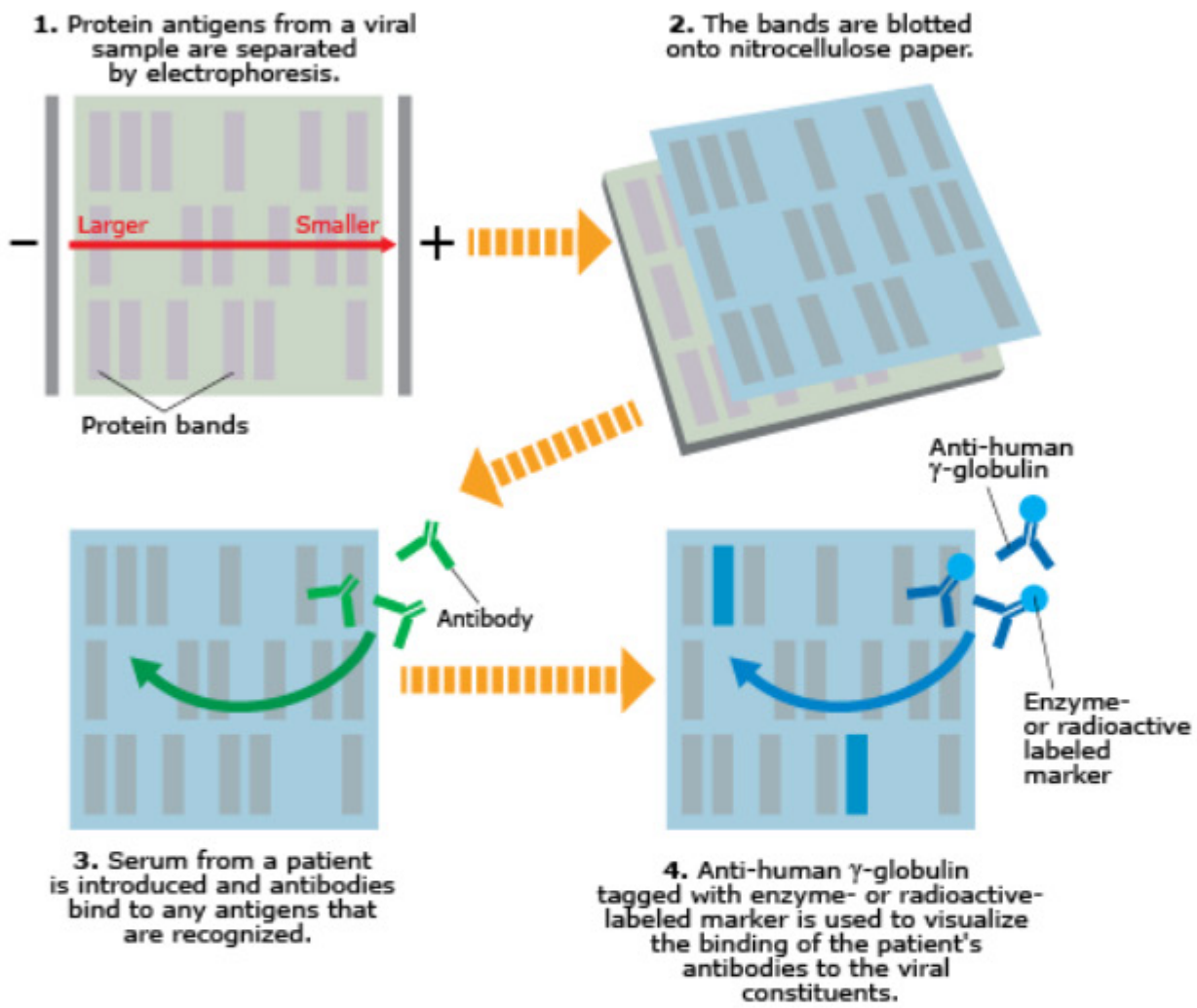
1.1.7 Western Blots

In this technique, a protein mixture is first separated by electrophoresis. Next, the proteins are transferred (blotted) to a nitrocellulose membrane. The membrane is then incubated with an antibody that binds specifically to a protein of interest. This antibody, or a secondary antibody that binds it, may be linked to an enzyme that produces a colored or a radioactive product that can be detected as a band on x-ray film. Western blots are used as confirmatory assays in the diagnosis of HIV infection and Lyme disease.



▲ **Figure 6–1.1H** Western Blot Test

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1.2 Polymerase Chain Reaction

The polymerase chain reaction (PCR) is a technique that can amplify—make many copies of—a specific segment of DNA through a process similar to replication.

PCR requires four components:

1. A DNA template.
2. Two specific primers that are antiparallel and complementary to the DNA sequence flanking the interval of interest. These two primers bind opposite strands and face each other across this interval.
3. Nucleotide triphosphates.
4. DNA polymerase—usually a thermostable polymerase derived from thermophilic bacteria.

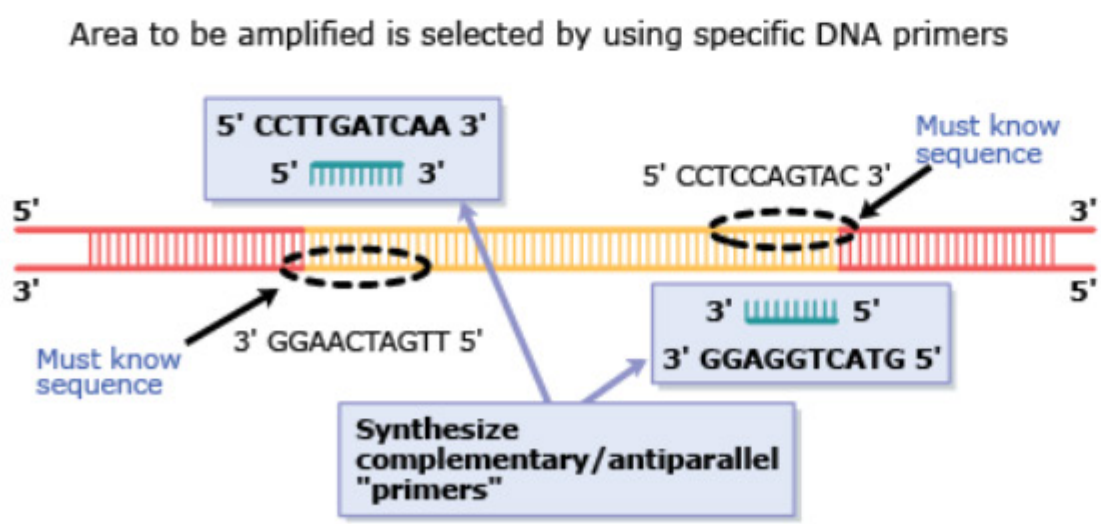
PCR is a three-step process:

1. The DNA is denatured (strands separated) by heating to 95°C.
2. The primers are allowed to anneal to the DNA by lowering the temperature to 55°C to 65°C.
3. The temperature is raised to 72°C, the temperature at which the bacterial DNA polymerase is most active, to allow polymerization of DNA starting at each primer.

This three-step process is then repeated for a number of cycles (usually 20 to 40). The amount of DNA produced grows exponentially, resulting in many copies of the region of interest.

Uses of PCR include:

- Identification of a sequence in a patient sample: e.g., diagnosis of infectious diseases by identification using primers that bind to and amplify the nucleic acid sequences of viruses and bacteria.
- Quantification of sequences: e.g., RT-PCR in HIV viral load analysis.
- Genotyping of short tandem repeat polymorphisms.
- Recombinant DNA applications.



▲ **Figure 6–1.2A** Polymerase Chain Reaction

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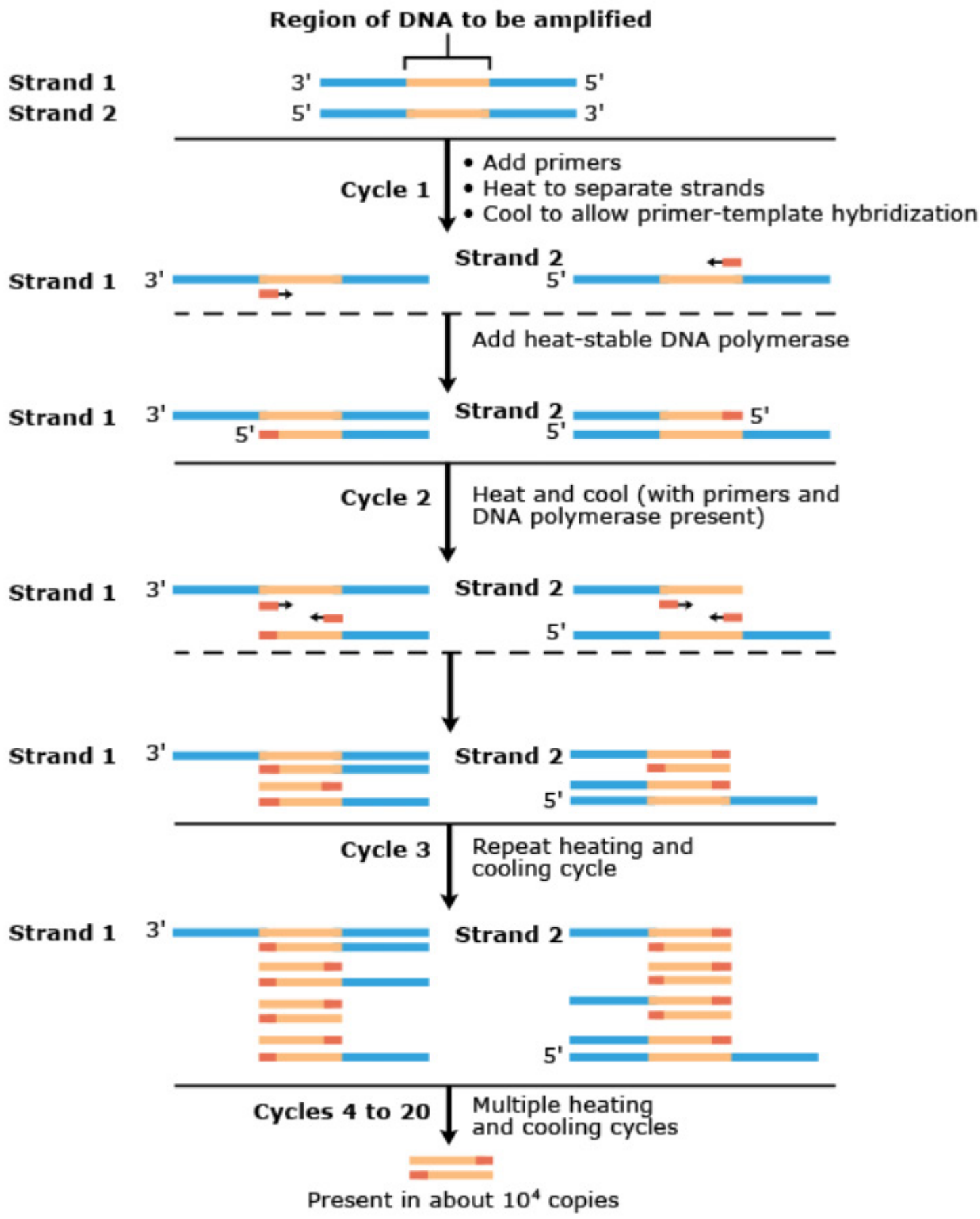

▲ **Figure 6–1.2B** DNA Segment Amplication by PCR Cycles

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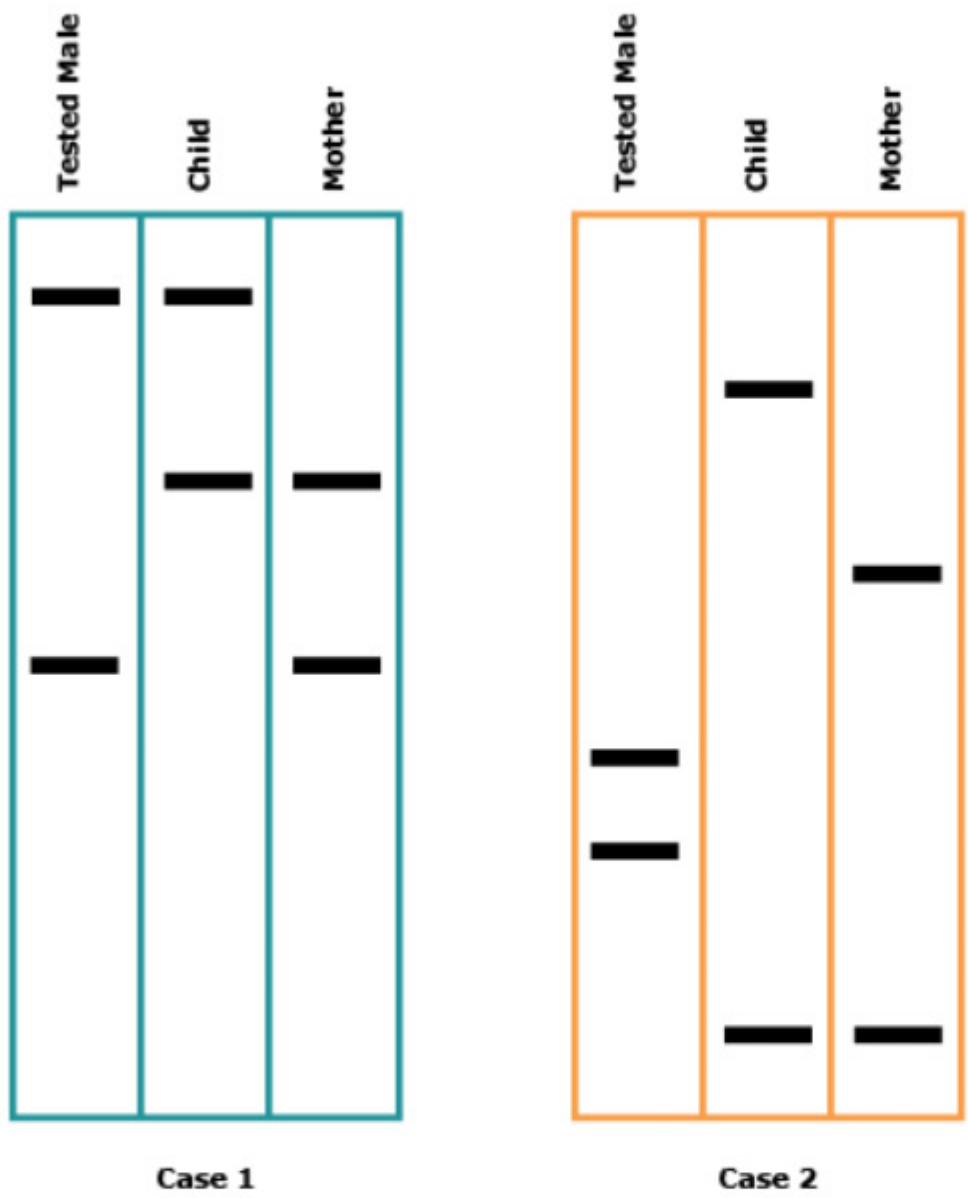
1.2.1 Genetic Fingerprinting

When repetitive sequences (Short Tandem Repeat Polymorphisms, or STRPs) occur outside of coding regions, variable expansions of these sequences do not affect the function of any genes. STRPs become highly polymorphic in populations and can be used in paternity testing and forensic medicine to develop a genetic fingerprint. Consider figure 6–1.2C: In case 1, the tested male could be the father of the child, whereas in case 2, the tested male could not be the father of the child because neither of his bands is shared with the child.



▲ **Figure 6–1.2C** Paternity Testing Using PCR

Connection to Genetics

STRPs are 2–6 bp segments that occur between exons of the genome. These segments can be digested by restriction enzymes to create a unique pattern of fragments that can be used to uniquely identify an individual.

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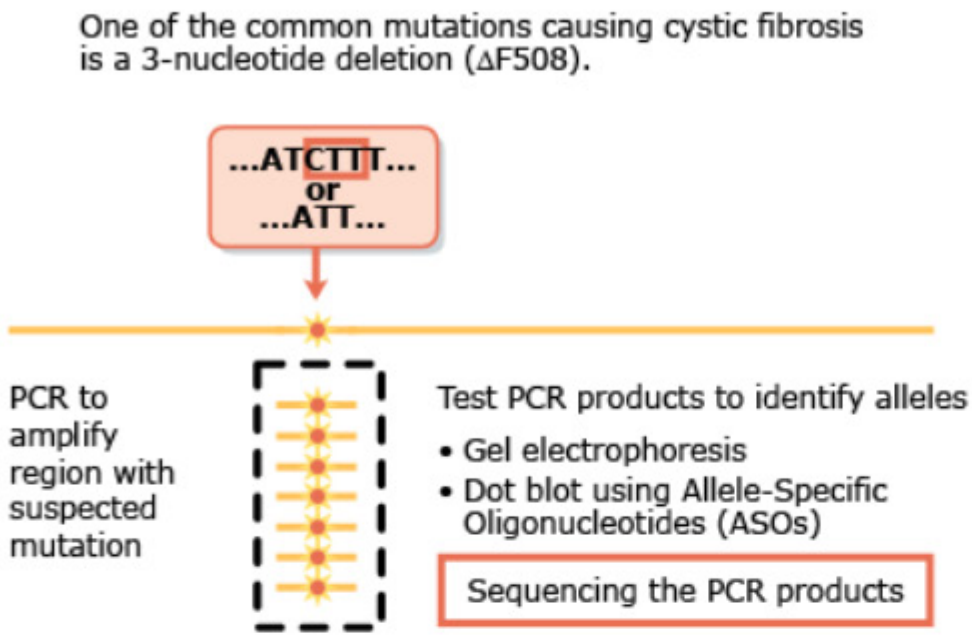
1.2.2 PCR in Direct Mutation Testing

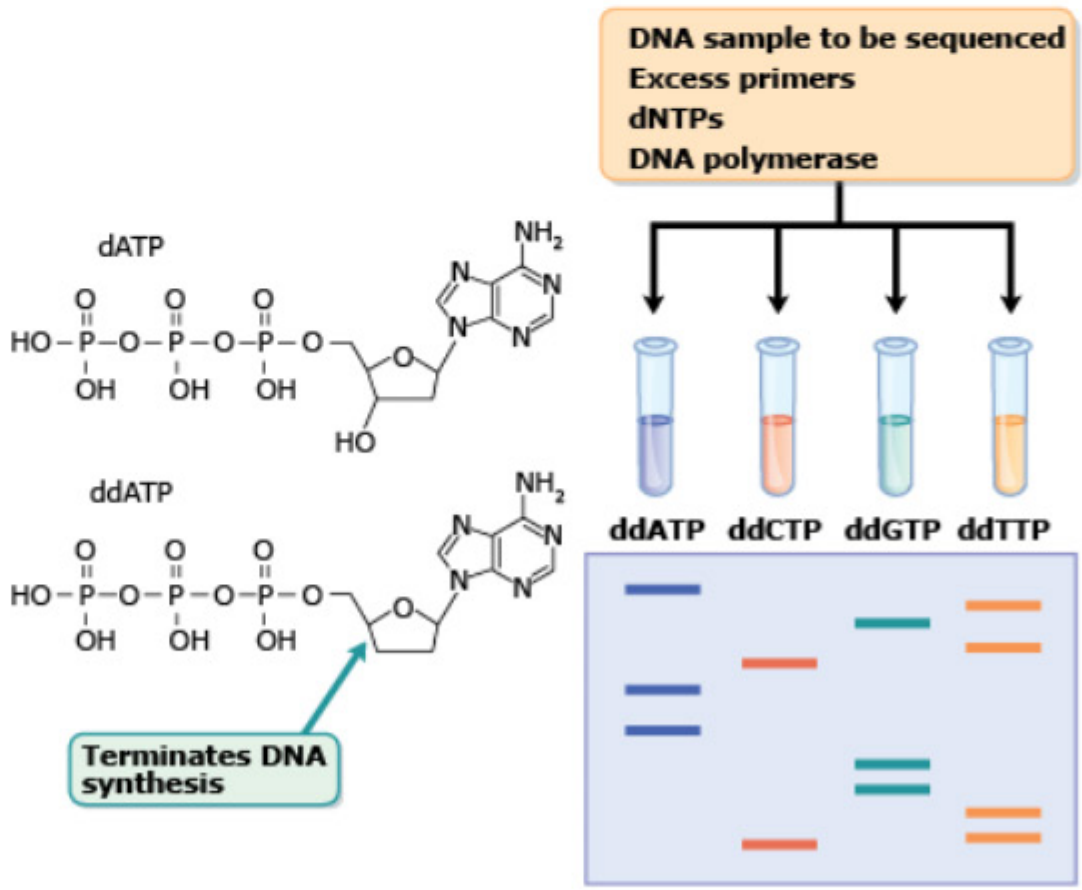
Amplification of known loci of disease genes can be used in direct mutation testing.



▲ **Figure 6–1.2D** PCR in Direct Mutation Testing

1.2.3 Sequencing DNA for Mutation Testing

If the location of a mutation is known, PCR can be used to amplify the region and sequence one of the strands to determine whether it contains the mutation. A sample of the DNA to be sequenced is put in each of four reaction mixtures containing DNA polymerase and deoxyribonucleotide triphosphates sufficient to synthesize new DNA. In each tube, a different dideoxynucleotide triphosphate (ddNTP) lacking 3' and 2' hydroxyl groups is added. These can be inserted into a growing chain of DNA but further elongation becomes impossible. The pieces of newly synthesized DNA in the tubes are separated by gel electrophoresis, and the sequence of the new strand can be read from the smallest to the largest fragments.



▲ **Figure 6–1.2E** DNA Sequencing

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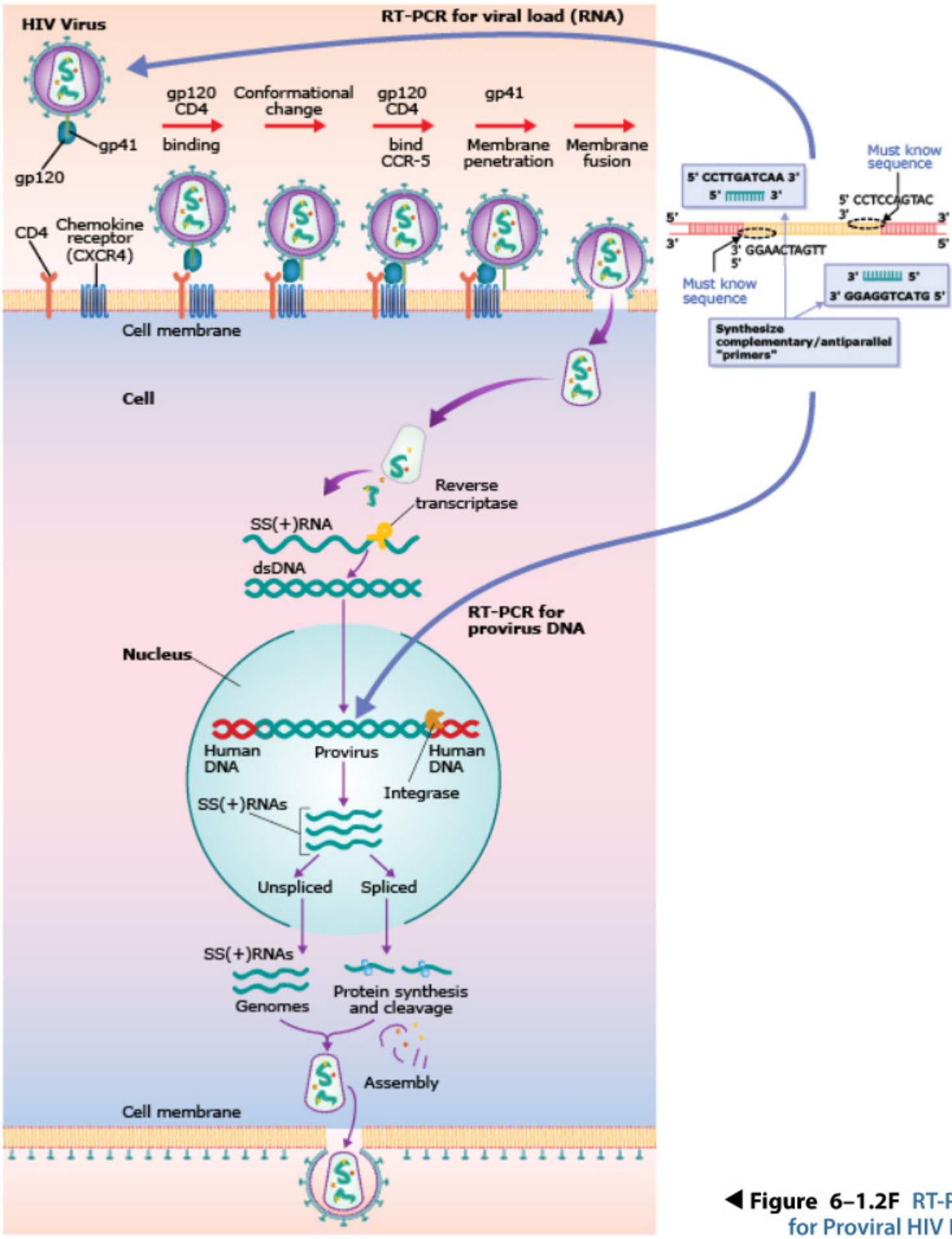
1.2.4 PCR in HIV Testing

PCR is used in HIV testing for cases in which the standard antibody screen (enzyme immunoassay, or EIA) and confirmation (Western blot) may not be sufficient. This test detects proviral DNA integrated into the chromosomes of the patient, providing a positive result much earlier than tests that depend on antibody production. PCR also provides a mechanism for neonatal HIV testing, as antibody tests are inconclusive due to maternally-derived transplacental IgG.



◀ **Figure 6–1.2F** RT-PCR Testing for Proviral HIV DNA

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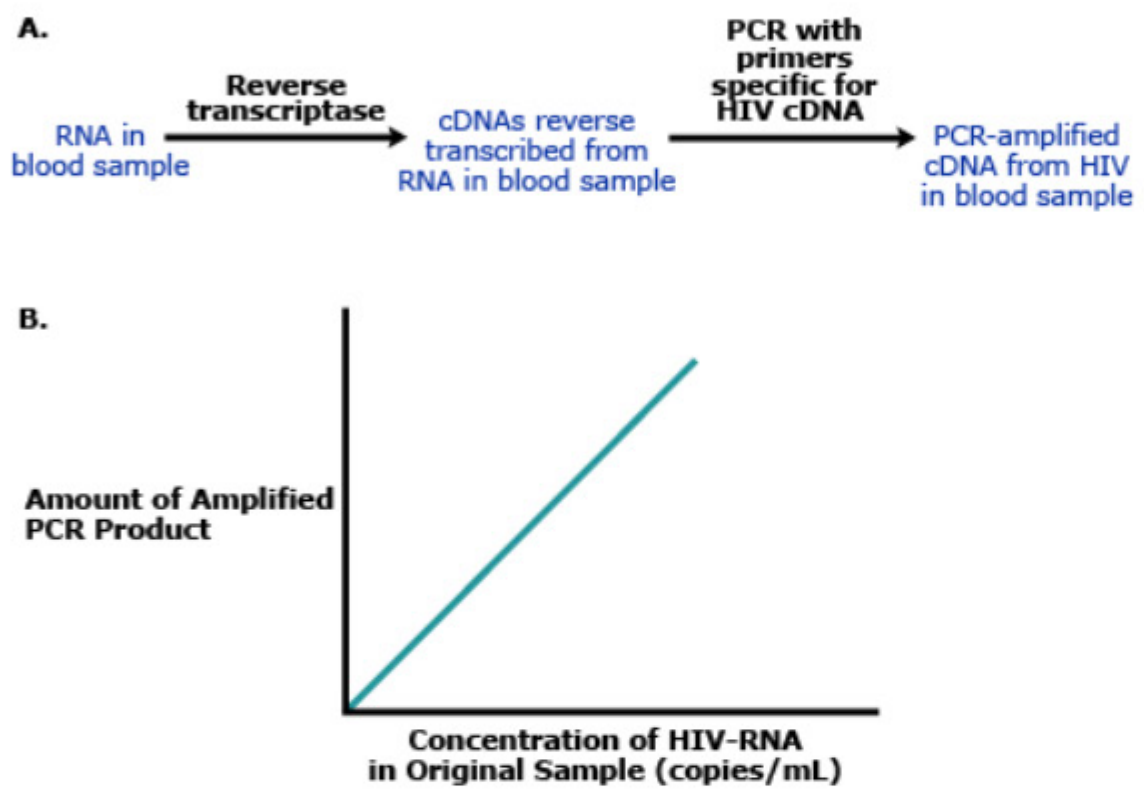
1.2.5 Reverse Transcriptase PCR

Reverse transcriptase PCR (RT-PCR) detects RNA in a sample and is a useful test to detect RNA virus infections. In HIV infections, RT-PCR is used to measure the viral load: the concentration of virions circulating in the blood.

In this test, a blood sample is treated with reverse transcriptase to produce cDNA, which is then PCR amplified using primers for the end sequences of the viral genome. The amplified product is then measured and compared to a standard curve to determine the original amount (copies/mL) of viral RNA present.



▲ **Figure 6–1.2G** RT-PCR: Assessment of HIV Viral Load

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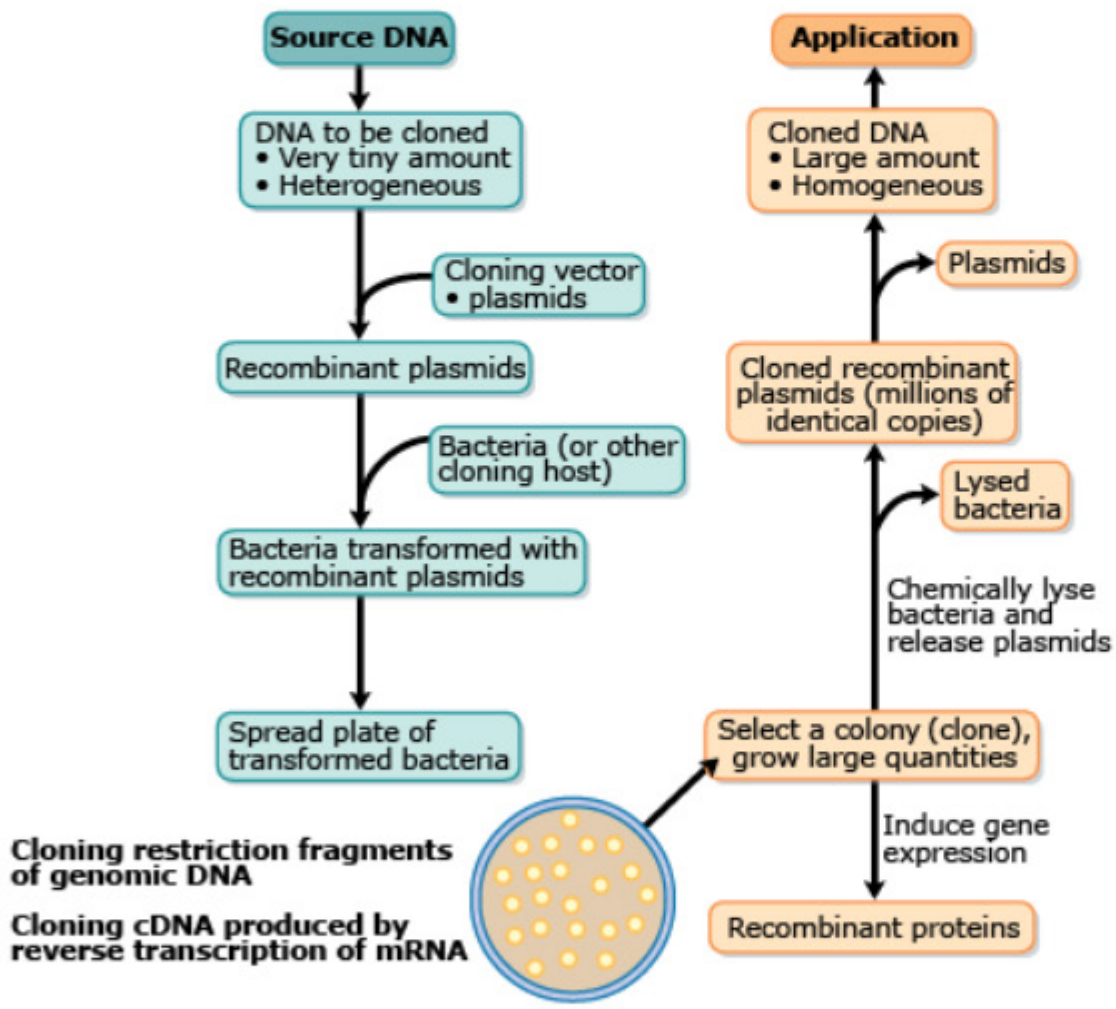
2 Recombinant DNA Technology

2.1 Overview of Recombinant DNA Technology

Recombinant DNA technology is used to provide reagents for genetic testing, for gene therapy, and for the production of specific proteins in large quantities. Any DNA fragment can be joined in vitro with the DNA of a self-replicating vector.

The steps for DNA cloning and isolation of cloned material include:

- Ligate DNA of interest into a vector DNA molecule capable of autonomous replication (e.g., a plasmid).
- Allow the recombinant vectors to be taken up by host cells (e.g., bacteria or yeasts).
- Isolate individual colonies of the host cells (clones with a single recombinant vector) and grow large quantities.
- Lyse the host cells and re-isolate the recombinant vectors.
- Remove the cloned DNA from the vector.



▲ **Figure 6–2.1** Cloning Recombinant DNA

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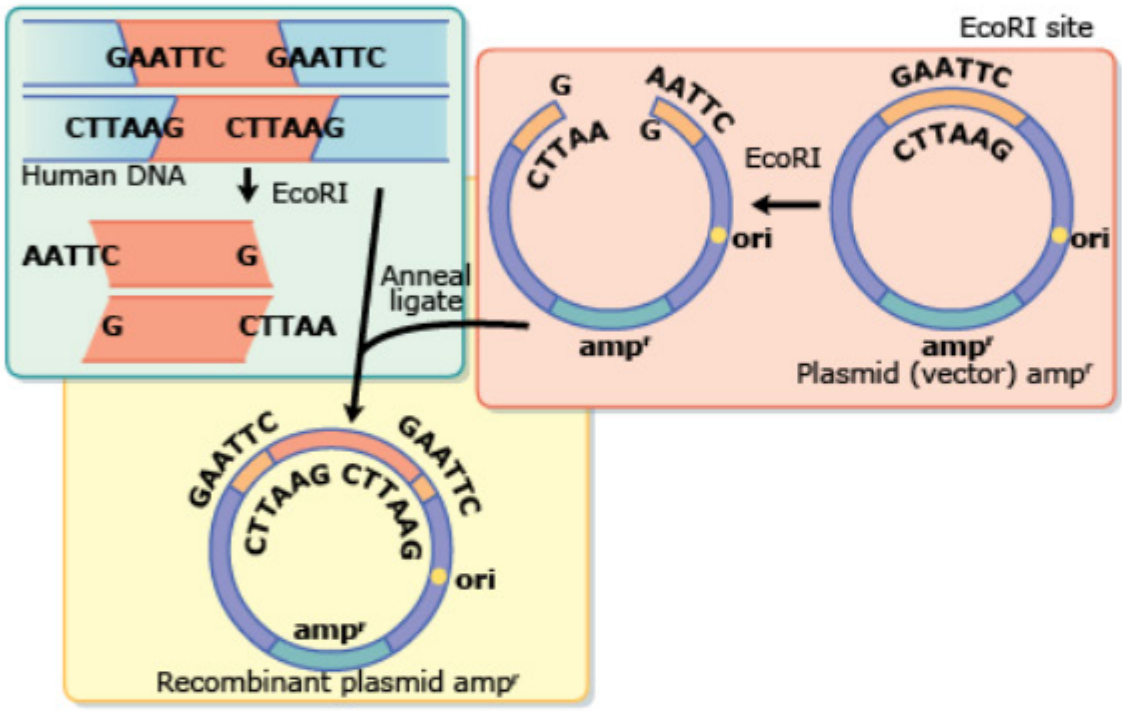
2.2 Cloning Restriction Fragments Using Vectors

A vector is a piece of DNA (viral or yeast chromosome, plasmid) with the capacity of autonomous replication. Vectors must have at least one restriction endonuclease site, an origin of autonomous replication, and an antibiotic resistance gene, which will allow for selection of clones containing the recombinant DNA.

The vector is cut with the restriction endonuclease and mixed with the restriction fragments to be cloned. DNA ligase is used to form phosphodiester bonds between the vector and the fragment. The recombinant vectors are now added to host cells, and cells that have taken up the appropriate recombinant vectors are identified by their antibiotic resistance. A complete collection of vectors produced in this way that represents the total genomic DNA of a single organism is referred to as a *genomic DNA library*.



▲ **Figure 6–2.2** DNA Production of a Recombinant Plasmid

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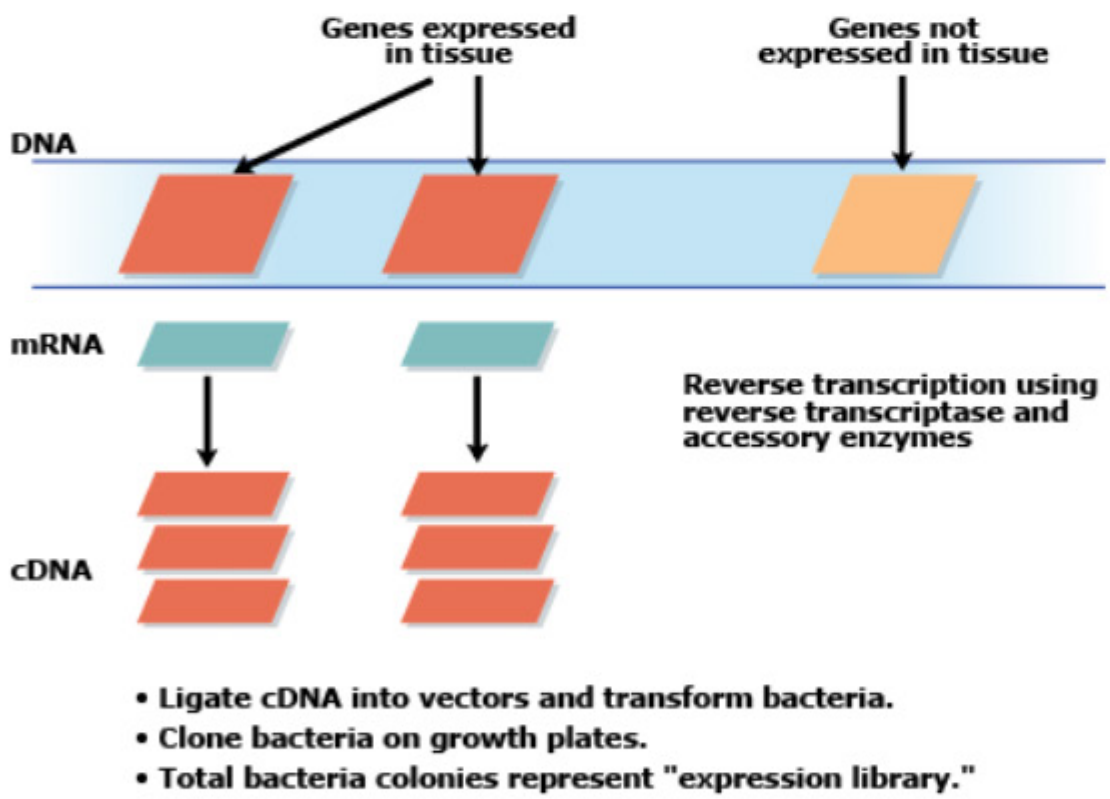
3 Cloning Genes Using Reverse Transcription

If the goal of gene cloning is to have that gene expressed inside a host cell, the entire coding sequence must be intact. Additionally, if a eukaryotic protein is to be expressed in a prokaryotic cell, it cannot contain introns that could not be processed in a bacterial cell. For these cases, it is often more appropriate to clone cDNA rather than DNA restriction fragments.

3.1 Producing cDNA by Reverse Transcription of mRNA

Messenger RNA from cells known to express a desired gene is treated with reverse transcriptase to produce double-stranded cDNA, which is then recombined in a vector as above. The advantages of this procedure include:

- All genes expressed will be cloned with the desired gene.
- Non-expressed DNA will not be cloned.
- Each cDNA is the complete coding sequence of a gene.
- cDNAs will not contain introns.
- The cloning procedure culminates in the production of an expression library.



▲ **Figure 6–3.1** Cloning Expressed Genes by Producing cDNAs

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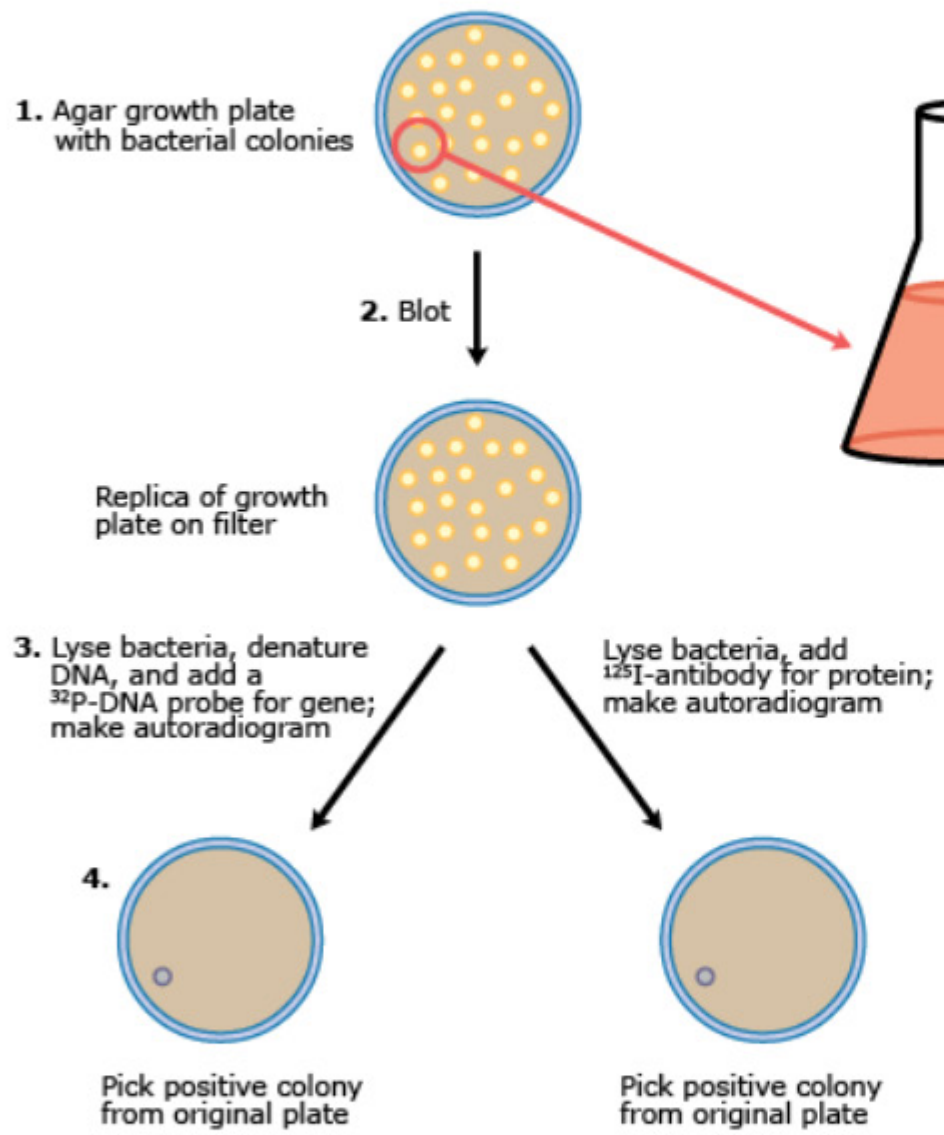
3.2 Expression Libraries

The cloned cDNA libraries (expression libraries) can be used to:

- Produce recombinant proteins (factor VIII, insulin, HBsAg).
- Sequence specific genes and identify mutations.
- Produce genes for gene therapy.
- Produce transgenic animals.

3.3 DNA Libraries

Screening of DNA libraries is performed by blotting colonies from the culture plate, lysing them, and treating them with a radioactive probe specific for the DNA sequence (for DNA libraries) or protein product (for expression libraries). An autoradiogram of the treated blot is produced and the radioactive colony is identified.



▲ **Figure 6–3.3** Screening a DNA Library

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4 Applications of Gene Cloning

4.1 Production of Recombinant Proteins

If the goal of cloning is the production of recombinant protein, then appropriate sequences for transcription and translation must be provided. For example, to produce human proteins in bacteria, a bacterial promoter and Shine-Dalgarno sequence must be near the insertion site for the cDNA.

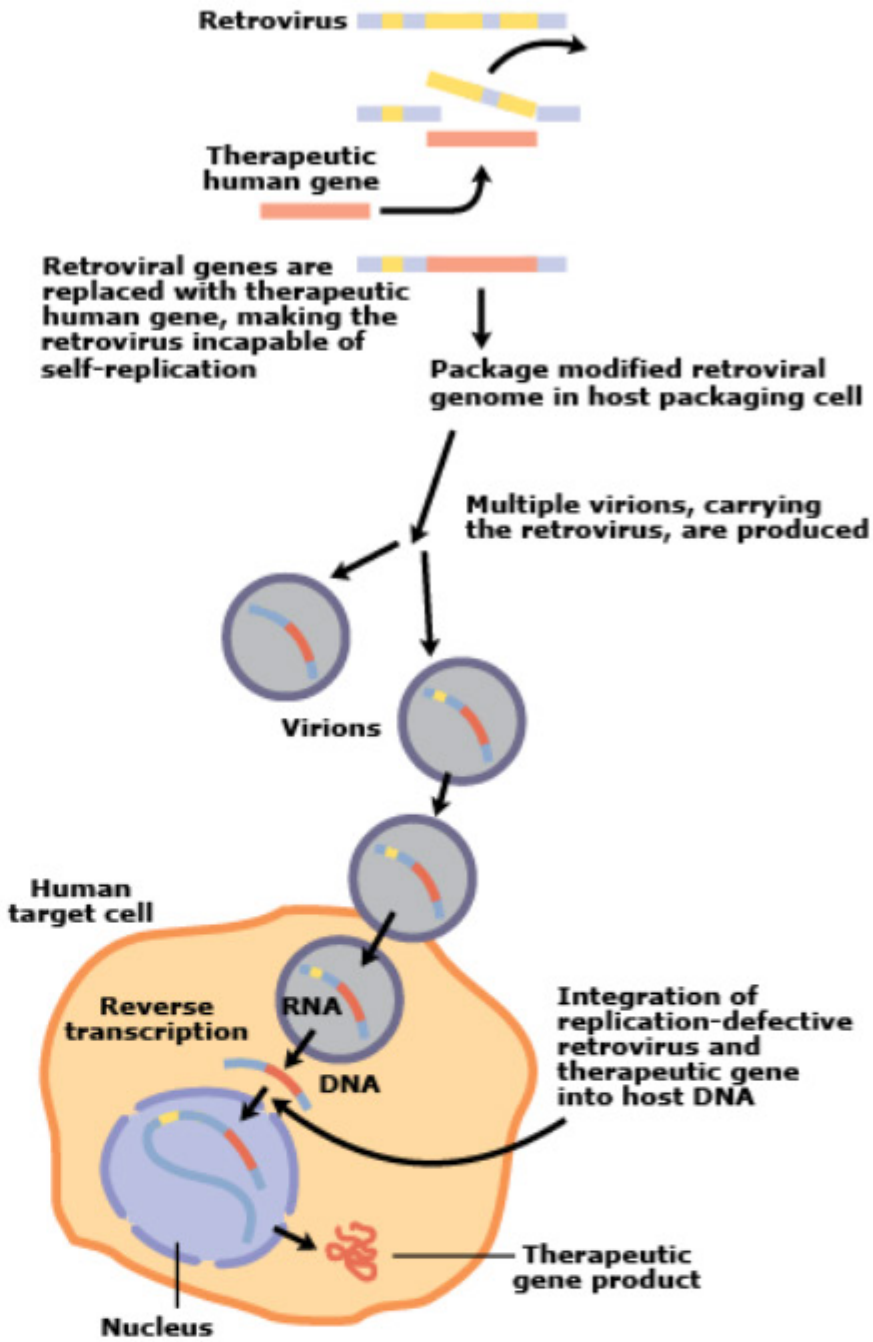
4.2 Gene Therapy

The goal of somatic cell gene therapy is the introduction of a replacement normal gene into the cells of individuals with inherited diseases. This has been undertaken in the treatment of X-linked severe combined immunodeficiency but unfortunately the beneficial result was not lasting.

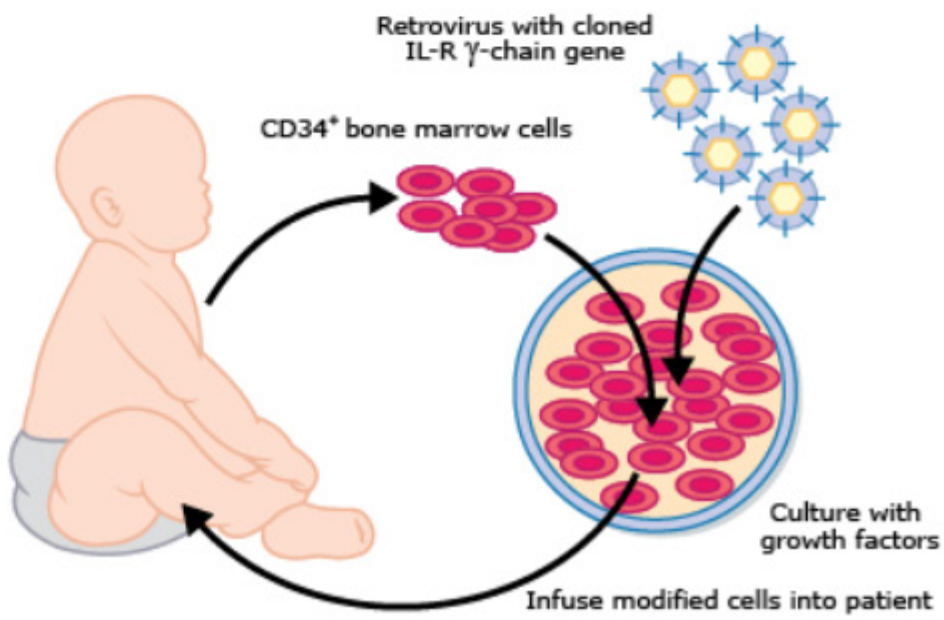
In order to introduce cloned genes for the purposes of gene therapy, a delivery vector must be used. Most delivery vectors are viruses that have been modified so the virus can infect the cell but not complete its replication cycle.

Retroviruses naturally insert their reverse transcribed DNA into the chromosomes of target cells. Cells must be actively replicating for successful integration, and because the virus genome integrates randomly, it is possible for insertion near a cellular oncogene with the result of oncogenesis.

Adenoviruses have the advantage that they can infect nondividing cells, but the disadvantage is that their DNA is not integrated into the chromosomes and therefore eventually is lost.



▲ **Figure 6–4.2A** Retroviral Gene Therapy



▶ **Figure 6–4.2B** Somatic Gene Therapy for X-Linked Severe Combined Immunodeficiency

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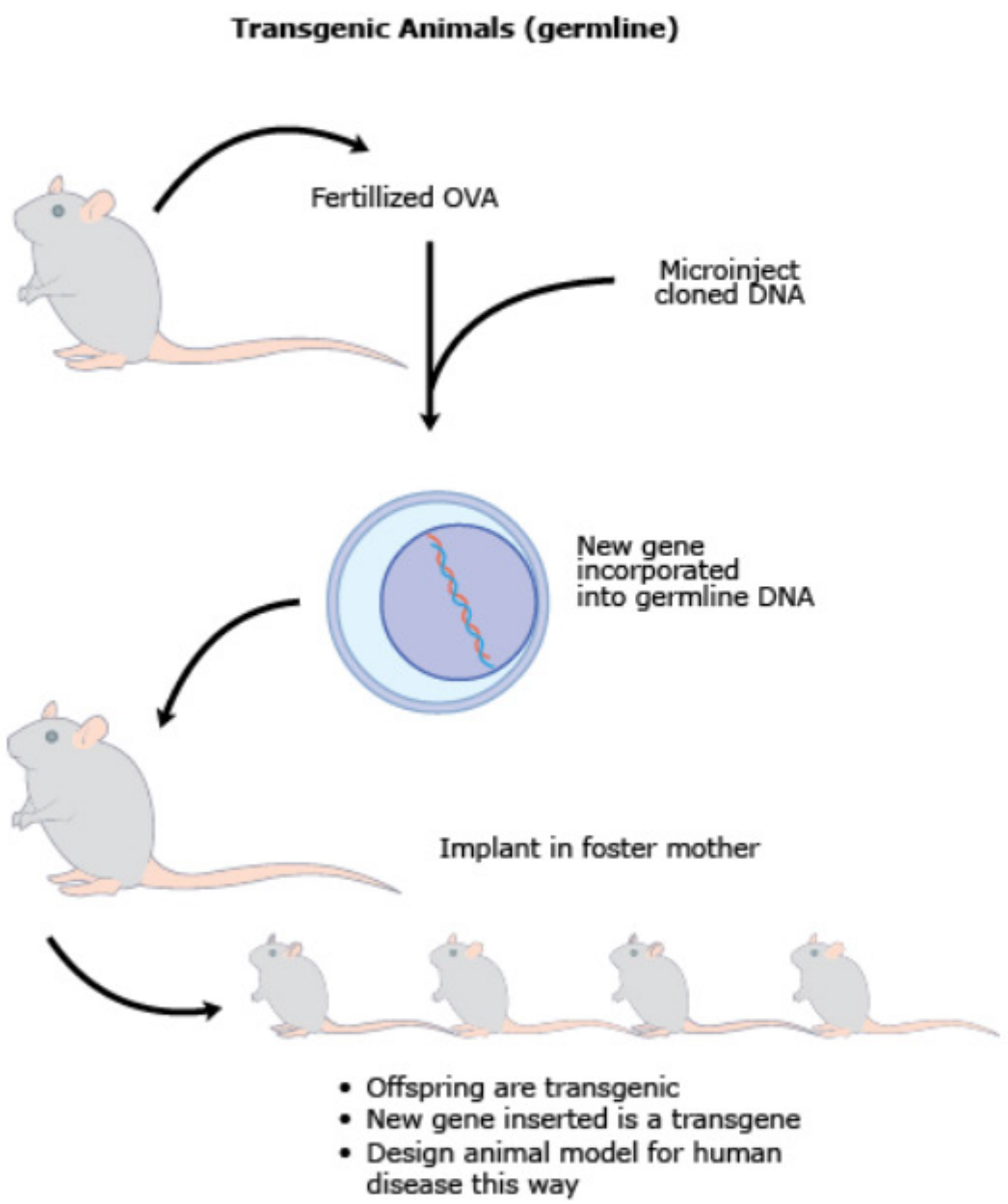
4.3 Transgenic Mice

If a cloned gene is introduced into a fertilized ovum or embryonic stem cell, a laboratory animal can be produced that can be a model for human disease. The introduced gene is called a *transgene*. If the process is used to intentionally delete a gene, the result is referred to as a *knockout animal*.

4.3.1 Introduction of Cloned Genes Into a Fertilized Ovum

If the transgene incorporates into the nuclear DNA it will be expressed. The ova are implanted into a surrogate mother, and the resulting offspring may contain the transgene in all of their cells.



▲ **Figure 6–4.3A** Incorporation of Cloned DNA Into Mice

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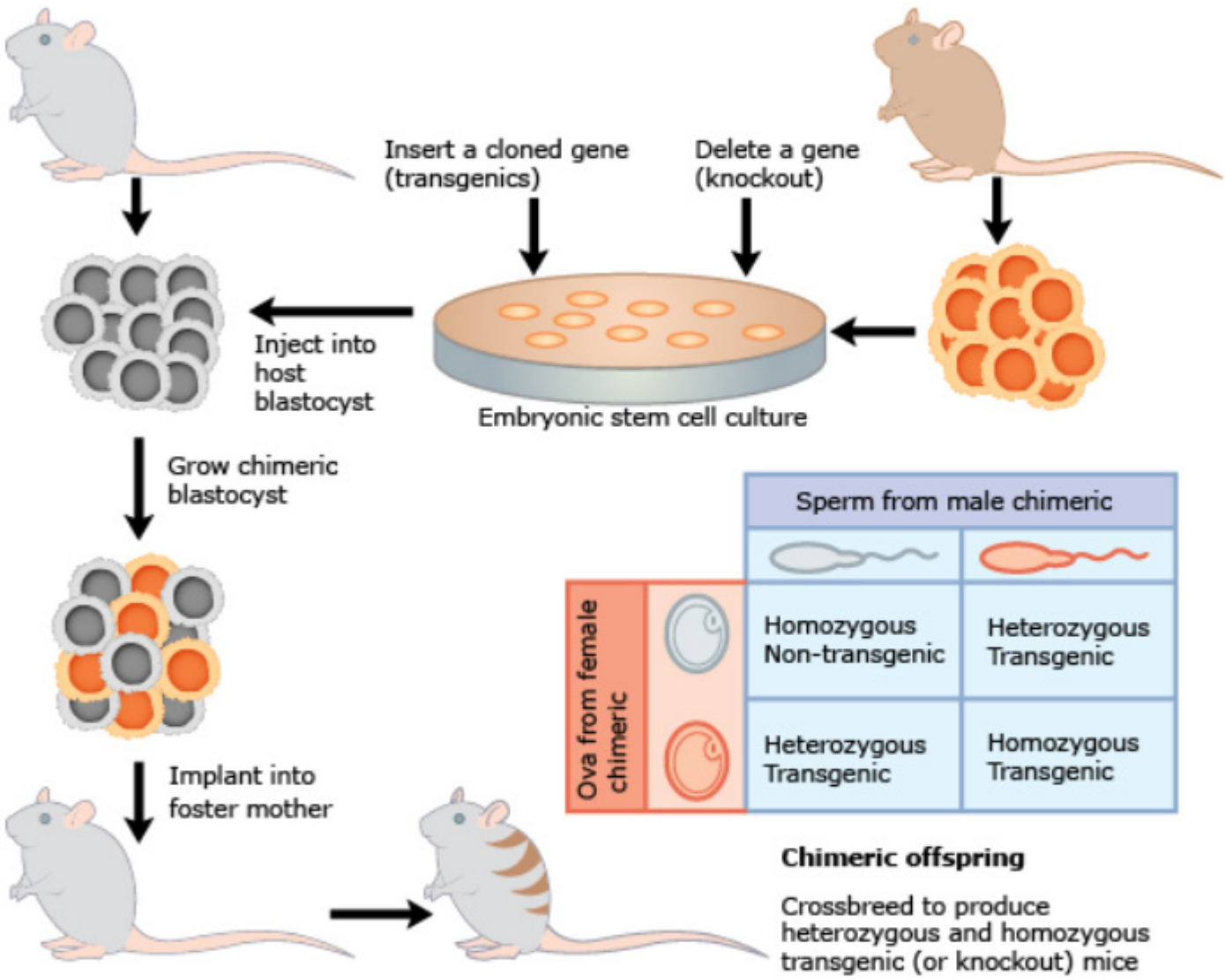
4.3.2 Introduction of Cloned Genes Into Embryonic Stem Cells

The introduction of cloned genes into cultures of embryonic stem cells has several advantages. The gene introduction can be performed in culture, and cells that have had the genes successfully inserted can be selected. Dominant and recessive alleles can both be studied since homologous recombination can be used to replace the existing copies of the gene with the cloned gene. The altered cells are injected into blastocysts and implanted into surrogate mothers. This creates a *chimeric mouse* consisting of germ cells from two cell lineages. These chimeras are then bred to create mice that are homozygous and heterozygous for the transgene.



▲ **Figure 6–4.3B** Producing Transgenic or Knockout Mice Using Embryonic Stem Cells

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Thermodynamics and Enzyme Kinetics

1 Thermodynamics

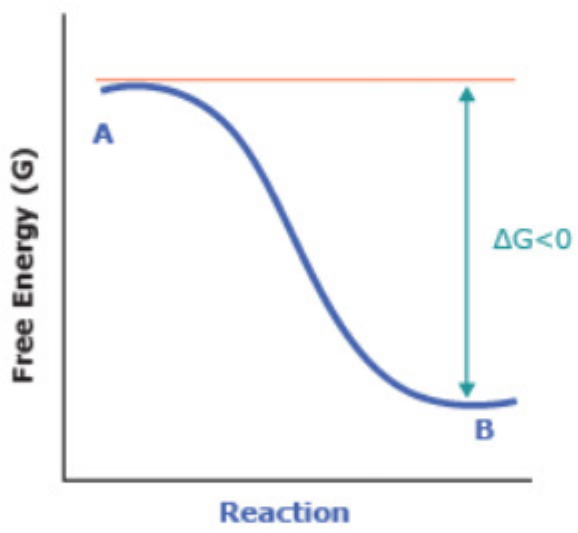
Thermodynamics is the study of energy movement between systems. Within the field of biochemistry, thermodynamics concerns the energy needed to drive biochemical reactions at the cellular level.

1.1 Energy

Cells require energy to perform all their necessary functions. This energy is obtained through chemical reactions. The amount of free energy (G) produced or consumed by a chemical reaction is known as ΔG, or the change in free energy. ΔG is calculated as $G_{product} - G_{substrate}$. This value tells us whether a reaction is spontaneous, that is, whether the reaction occurs without any exogenous input of energy.

1.2 Exergonic Reactions

An exergonic reaction, one in which $\Delta G < 0$ (negative), occurs spontaneously. This is an energy-producing reaction. Figure 7–1.2, an energy diagram, shows the conversion of a substrate with high free energy to a product with low free energy. Since $G_{product} < G_{substrate}$, ΔG is negative.



▲ **Figure 7–1.2** Exergonic Reaction

USMLE® Key Concepts

For Step 1, you must be able to:

- Describe the principles of thermodynamics and the role of ATP in driving biochemical reactions.
- Define the functions of enzymes in biochemical reactions and the role of different types of inhibitors in modulating these functions.
- Solve kinetic problems using the Michaelis-Menten and Lineweaver-Burk equations.

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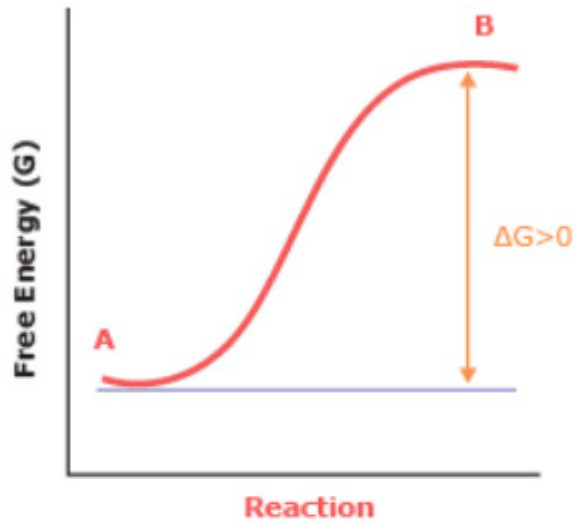
1.3 Endergonic Reactions

An endergonic reaction, one in which $\Delta G > 0$ (positive), requires an input of free energy to occur. This is an energy-consuming reaction. Figure 7–1.3 shows the conversion of a substrate with low free energy to a product with high free energy. Since $G_{product} > G_{substrate}$, ΔG is positive.



▲ **Figure 7–1.3** Endergonic Reaction

For reversible reactions, ΔG determines the direction of the reaction. For reaction $A \rightarrow B$, if $\Delta G < 0$, the reaction will be $A \rightarrow B$. If $\Delta G > 0$, then the reaction will be $B \rightarrow A$.

1.4 ATP-Coupled Reactions

Most biosynthetic reactions in the cell involve making something more complex from simpler building blocks (e.g., DNA replication, protein synthesis, etc.). These are endergonic reactions that require energy.

The cell drives these endergonic reactions by coupling them with exergonic reactions such as the hydrolysis of high-energy phosphates from ATP. For example, think about the formation of a peptide bond:

$$AA_1 + AA_2 \rightarrow AA_1\text{–}AA_2,\ \Delta G > 0$$

This is coupled to ATP hydrolysis:

$$ATP \rightarrow ADP + P_i,\ \Delta G < 0$$

The net reaction is exergonic:

$$AA_1 + AA_2 + ATP \rightarrow AA_1\text{–}AA_2 + ADP + P_i,\ \Delta G < 0$$

Important Concept

Spontaneous: $\Delta G < 0$

Non-spontaneous: $\Delta G > 0$

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1.5 ΔG and Substrate Concentrations

ΔG is also related to substrate concentration according to the following equation:

$$\Delta G = \Delta G^{\circ} + RT \times ln \left(\frac{[B]}{[A]}\right)$$

- ΔG° is the standard free energy change (ΔG when all substrates are at equal concentrations)
- R is the gas constant (1.987 cal / mol × K)
- T is the temperature (K)

With this equation we can see that even an endergonic reaction (A → B) can be spontaneous at high concentrations of substrate A.

Eventually reversible reactions (A ↔ B) will reach an equilibrium at which the rate of reaction A → B is equal to reaction B → A. The concentrations of substrate A and product B at equilibrium define the equilibrium constant, K_{eq}.

$$K_{eq} = \frac{[B]_{eq}}{[A]_{eq}}$$

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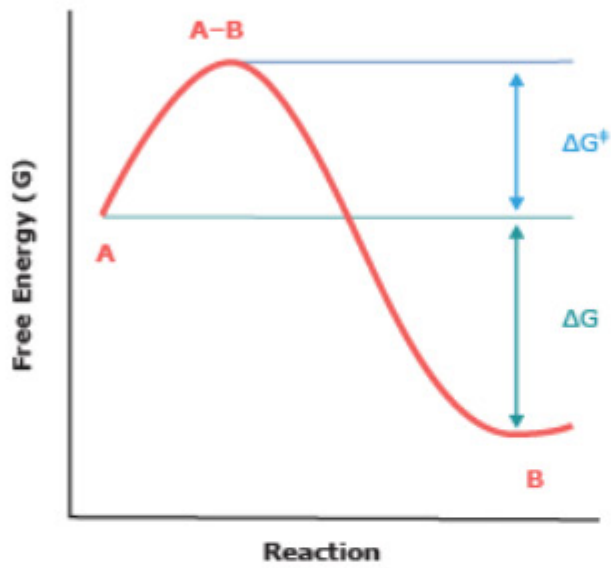
2 Kinetics

In biochemistry, kinetics is the study of reaction rates and their regulation by catalysts, particularly enzymes.

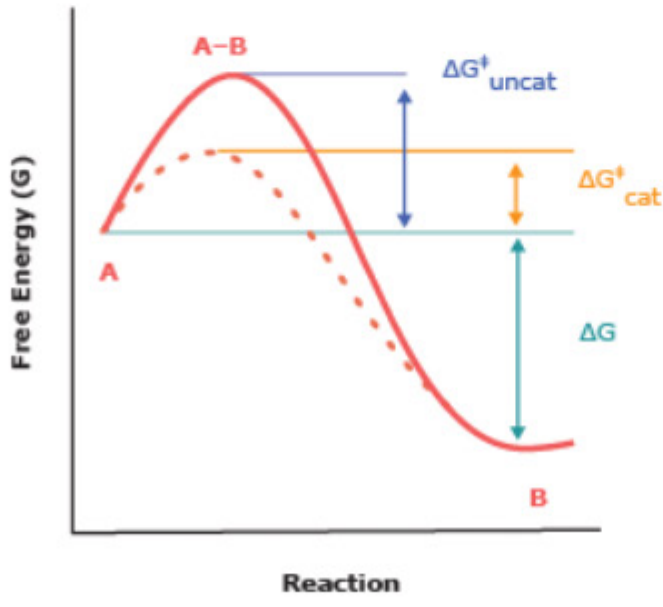
2.1 Enzymes as Catalysts

Most chemical reactions can be described as the conversion of a substrate to a product (A → B). This process often includes a *transition state* (A – B), an intermediate form between substrate and product. This intermediate usually has higher free energy (G) than the substrate. For the reaction to proceed, there has to be an input of energy to overcome this barrier—this is known as *the energy of activation* (ΔG‡). This need for energy affects the rate of the reaction; that is, the greater ΔG‡, the slower the reaction.



▲ **Figure 7–2.1A** Conversion of Substrate to Product

Catalysts increase the rate of reaction by lowering ΔG‡ for the reaction. This means that less energy is required to start the reaction, and therefore the rate of the reaction is increased.



▲ **Figure 7–2.1B** Conversion of Substrate to Product With Catalyst

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In biological systems, most catalysts are proteins called enzymes. These have several properties:

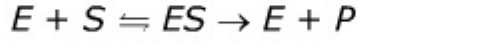
- Enzymes have high specificity for their substrates.
- Enzymes do not change the concentration of substrates and products at equilibrium (i.e., K_{eq}), but they decrease the time required to reach equilibrium.
- Enzymes are not permanently changed in the course of the reaction.

Some enzyme-related definitions:

- **Active Site:** The site in the three-dimensional protein structure at which the substrate binds and is converted to product.
- **Cofactor:** A non-protein chemical compound that associates with an enzyme and participates in catalysis. These can be nonorganic, like metal ions (e.g., Ca^{2+} or Mg^{2+}), or organic, like vitamins.
- **Coenzyme:** A loosely bound cofactor, often a vitamin or mineral, that freely associates and dissociates during catalysis (e.g., NADH or thiamine pyrophosphate).
- **Prosthetic Group:** A tightly bound cofactor that remains stably bound to the enzyme during the reaction.
- **Apoenzyme:** An inactive enzyme, without its cofactor.
- **Holoenzyme:** An enzyme with a bound cofactor.

2.2 Kinetic Analysis

Kinetics describes the rates of reactions, that is, how fast conversion of substrate to product occurs. Consider the following reaction, in which an enzyme (E) combines with a substrate (S) in a reversible reaction to form a transition state (ES), the complex between the enzyme and the substrate. This is then converted to product (P) and free enzyme.



$$E + S \rightleftharpoons ES \rightarrow E + P$$

Each of these steps has a rate constant, which expresses the activity of the enzyme, that is, the number of substrate molecules converted to product in a given time when the enzyme is saturated with substrate:

- k_1 = rate constant for $E + S \rightarrow ES$
- k_2 = rate constant for $ES \rightarrow E + S$
- k_3 = rate constant for $ES \rightarrow E + P$

2.3 Rate and Concentration

In most situations, the rate of enzyme-catalyzed reactions depends on the concentration of the substrates. This relationship hinges on the order of reaction:

- A first order reaction depends on the concentration of a single substrate.
- A second order reaction depends on the concentration of two substrates.
- A zero order reaction does not depend on substrate concentration. This is the condition when substrate concentrations are saturating.

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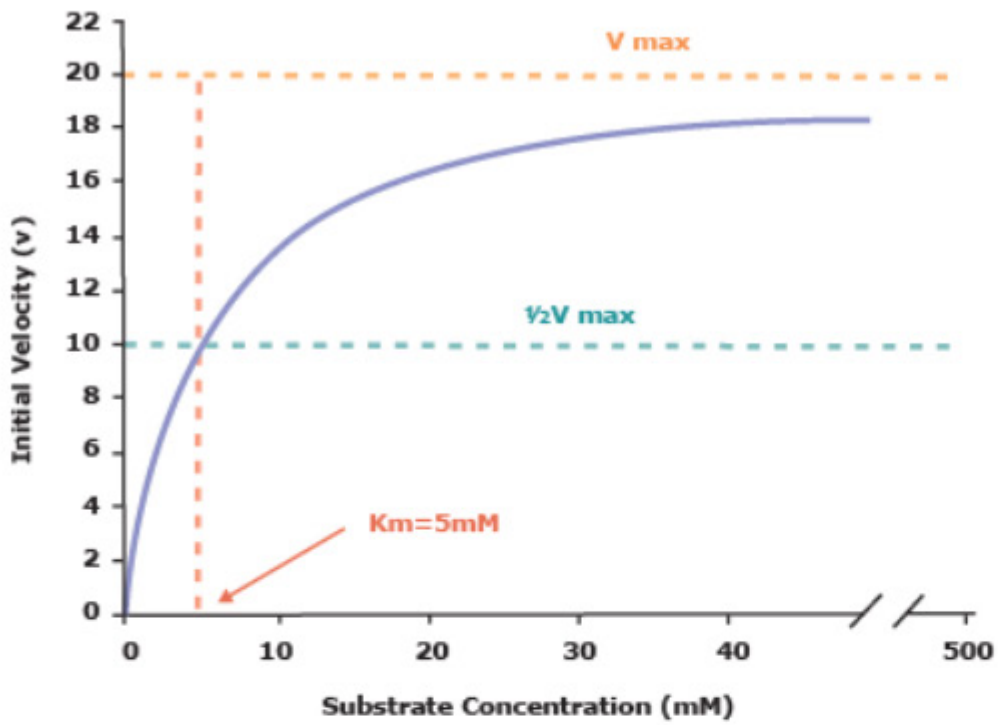
2.4 The Michaelis-Menten Equation

A first-order reaction can be graphed as rate (v) versus substrate concentration ([S]):



▲ **Figure 7–2.4** Michaelis-Menten Plot

For each reaction, there are two constants:

- V_{max}: the maximal reaction rate when the substrate is saturating.
- K_M: the Michaelis constant; the substrate concentration at which the rate is half maximal (= ½ V_{max}).

The Michaelis constant (K_M) is related to the affinity of the enzyme for its substrate:

- A small K_M indicates high affinity because a lower substrate concentration is required to half-saturate the reaction.
- A large K_M indicates low affinity because a higher substrate concentration is required to half-saturate the reaction.

The relationship between rate and substrate concentration in a first-order reaction is described by the Michaelis-Menten equation, which is also the equation of the graph above:

$$V = V_{max}\left(\frac{[S]}{[S] + K_M}\right)$$

2.5 The Lineweaver-Burk Plot

To determine K_M and V_{max} experimentally, the reaction rate is measured at various substrate concentrations. These can then be plotted on a graph and the equation derived.

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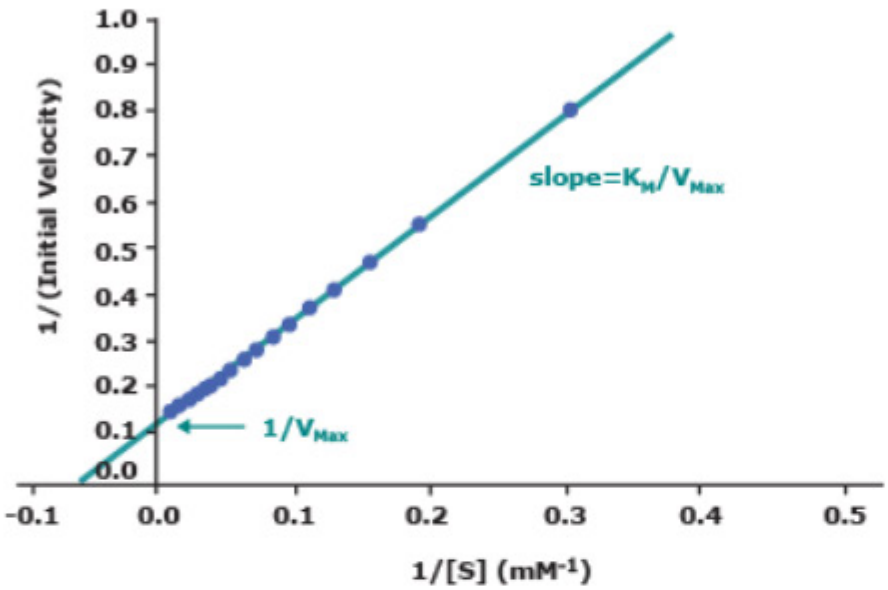
These calculations can be simplified by writing the Michaelis-Menten equation as a double reciprocal, such that the values on the X axis are 1/[S] and the values on the Y axis are 1/V. This is the Lineweaver-Burk equation:

$$\frac{1}{V} = \frac{K_M}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

This is an equation for a straight line in the form of y = mx + b, such that a Lineweaver-Burk plot looks like this:



▲ **Figure 7–2.5** Lineweaver-Burk Plot

In this equation:

- The slope is K_M/V_{max}
- The Y-intercept is $1/V_{max}$
- The X-intercept is $-1/K_M$

2.6 Regulation of Enzymes

In order for the body to function properly, it has to maintain *homeostasis*, which is the ability to maintain constancy of function in the face of changing environmental conditions. One way to maintain homeostasis is to regulate the activity of enzymes. The following are various mechanisms of regulating enzyme activity:

2.6.1 Reversible Inhibitors

Inhibitors are compounds, usually small molecules, which inhibit or reduce the activity of a given enzyme. A reversible inhibitor binds to enzymes through non-covalent interactions and is reversible; that is, the enzyme can have normal function restored if the inhibitor is removed. There are two types of reversible inhibitors, competitive and noncompetitive.

Competitive Inhibitors Competitive inhibitors are similar in structure to the enzyme substrate. They bind the enzyme active site without being converted to product. In doing so, they block access of the normal substrate to the enzyme. This increases the K_M of the enzyme, that is, it takes a greater substrate concentration to generate half-maximal enzyme activity. However, V_{max} does not change.

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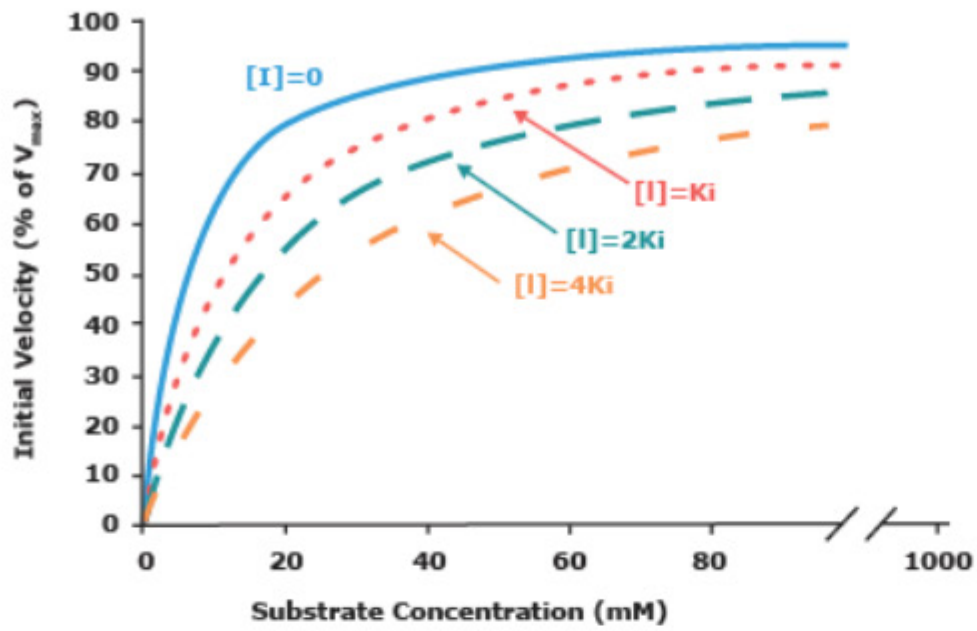
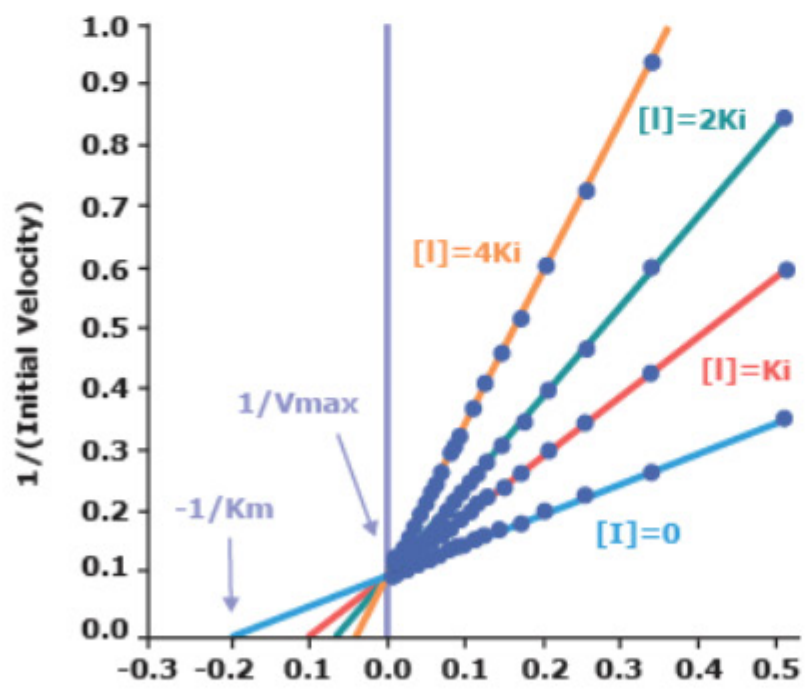
These changes are illustrated on the Michaelis-Menten plot:



▲ **Figure 7–2.6A** Competitive Inhibition (Michaelis-Menten)

With increasing concentrations of inhibitor, the Michaelis-Menten curve flattens and shifts to the right, meaning that the enzyme activity is lower for any given concentration of substrate. Thus, the apparent K_M of the enzyme, the substrate concentration at which the rate is half maximal, increases with increasing concentrations of competitive inhibitor. In contrast, the V_{max}, the maximal rate of the reaction does not change. The enzyme activity can still be maximized, given a high enough substrate concentration.

You can also see these changes in the Lineweaver-Burk plot:



▲ **Figure 7–2.6B** Competitive Inhibition (Lineweaver-Burk)

Remember that K_M increases with increasing concentrations of competitive inhibitor, but the V_{max} does not change. Since the slope of the line in the Lineweaver-Burk plot is calculated as K_M/V_{max}, a competitive inhibitor will increase the slope. The Y-intercept ($1/V_{max}$) does not change because V_{max} does not change.

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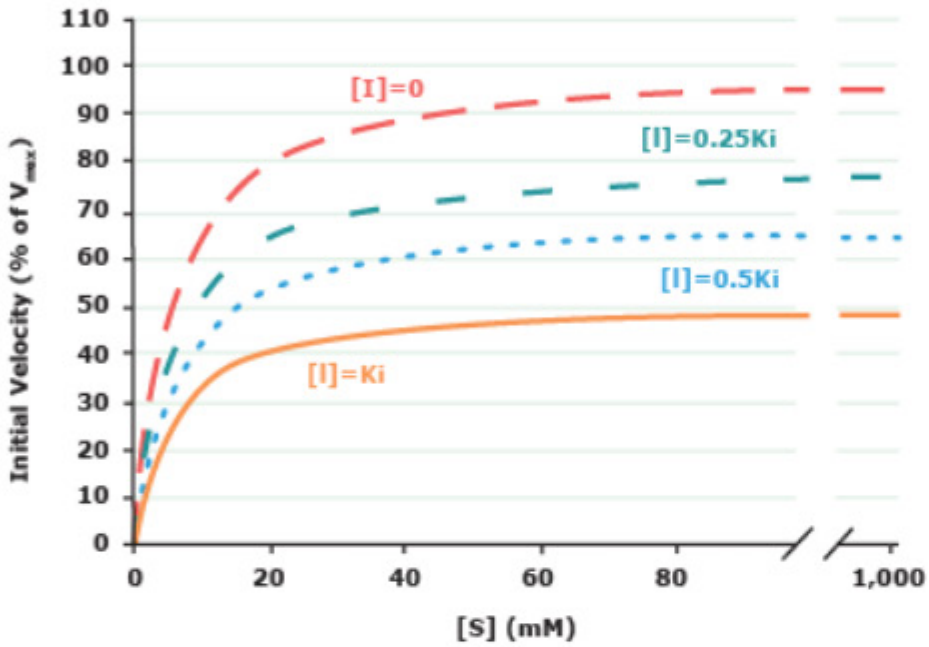
However, the X-intercept ($-1/K_M$) increases (gets closer to zero) with increasing K_M because the term $-1/K_M$ becomes a smaller and smaller negative number. The net result is a line that shifts upward, pivoting around the Y-intercept with increasing concentrations of competitive inhibitor.

Noncompetitive Inhibitors: Unlike competitive inhibitors, non-competitive inhibitors interact with the enzyme at another site (not the active site) and they affect the ability of the enzyme to catalyze the reaction. In doing so, they reduce the V_{max} of the enzyme. However, because the inhibitor is not competing with the substrate for access to the active site, there is no change in the K_M.

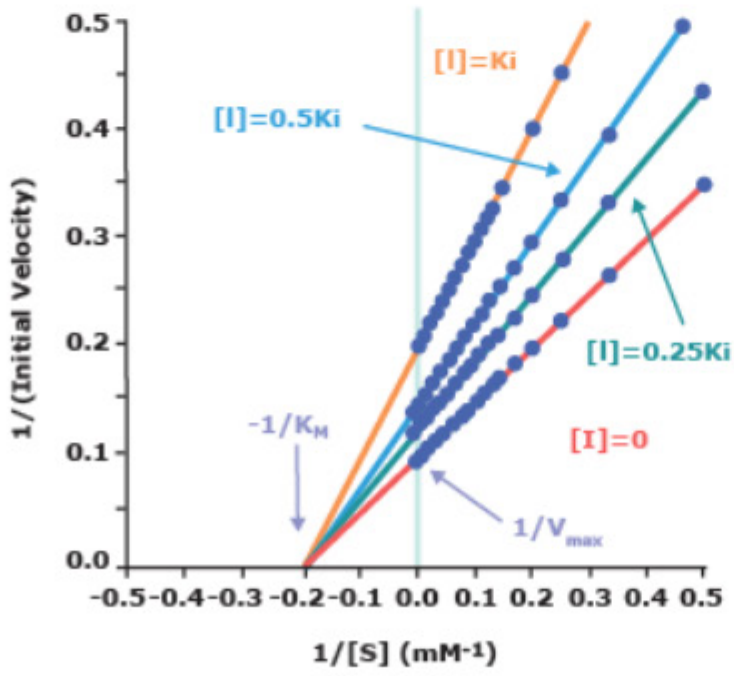
This is reflected in the Michaelis-Menten plot:



▲ **Figure 7–2.6C** Noncompetitive Inhibition (Michaelis-Menten)

Note the normal Michaelis-Menten curve in the absence of inhibitor. With increasing concentrations of noncompetitive inhibitor, there is a decrease in V_{max}, that is, the rate at which the curve reaches maximum is progressively decreased. However, K_M stays the same.

You can also see these changes in the Lineweaver-Burk plot:



▲ **Figure 7–2.6D** Noncompetitive Inhibition (Lineweaver-Burk)

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Remember that V_{max} decreases with increasing concentrations of noncompetitive inhibitor, but the K_M does not change. Because the slope of the line in the Lineweaver-Burk plot is calculated as K_M/V_{max}, a noncompetitive inhibitor will increase the slope as V_{max} (the denominator) decreases. For this reason, the Y-intercept ($1/V_{max}$) also increases as V_{max} decreases. The X-intercept ($-1/K_M$) does not change because K_M does not change. The net result is a line that shifts upward, pivoting around the X-intercept with increasing concentrations of noncompetitive inhibitor.

The following table is a summary of the impacts of competitive and noncompetitive inhibitors on the terms of the Lineweaver-Burk plot, slope, Y-intercept, and X-intercept.

▼ Table 7–3.1 Competitive vs. Noncompetitive Inhibition

	Competitive	Noncompetitive
Slope (K_M/V_{max})	↑	↑
Y-intercept ($1/V_{max}$)	—	↑
X-intercept ($-1/K_M$)	↑	—

Clinical Application

HIV Reverse Transcriptase Inhibitors

The availability of effective drugs to block replication of the human immunodeficiency virus (HIV) has dramatically improved the prognosis for infected individuals. One target of these drugs is the enzyme reverse transcriptase (RT). The HIV virus is a retrovirus, meaning that it uses RT to make DNA from an RNA template. Drugs called reverse transcriptase inhibitors (RTIs) bind to and inhibit the activity of this enzyme, preventing the virus from replicating. These can be divided into nucleotide/nucleoside analog RTIs (NRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). NRTIs, as their name implies, bind to the nucleotide-binding active site of the enzyme, and are therefore competitive inhibitors. NNRTIs on the other hand, bind to a separate site, away from the nucleotide-binding pocket, and are therefore noncompetitive inhibitors. Treatment of HIV commonly involves simultaneous and synergistic use of NNRTIs and NRTIs, reducing the likelihood that the virus will develop resistance to therapy through mutations at drug binding sites.

2.6.2 Irreversible Inhibition

Irreversible inhibitors permanently inhibit the function of an enzyme. The most common mechanism is covalent modification of an enzyme by the inhibitor. An example of this mechanism is the inhibitory effect of aspirin on cyclooxygenase. Aspirin (acetylsalicylate) binds to cyclooxygenase and transfers its acetyl group from salicylic acid to the hydroxyl group of a serine in the active site of the enzyme, blocking its activity.

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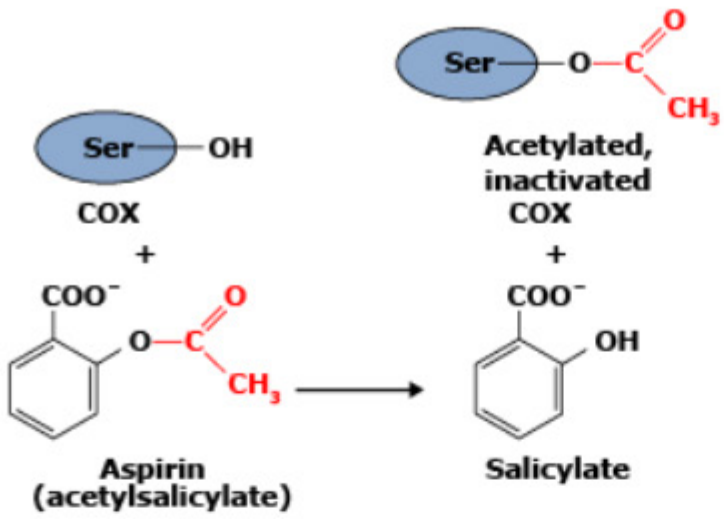


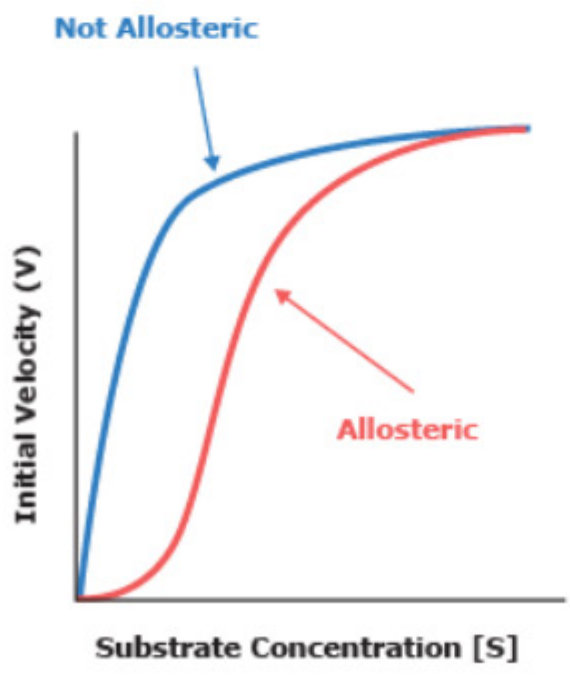
▲ **Figure 7–2.6E** Irreversible Inhibition: Aspirin and Cyclooxygenase

An important consequence of this mechanism is that the effect of aspirin is long-lasting since the only way to reverse its effects is to synthesize additional cyclooxygenase enzymes.

2.6.3 Allosteric Regulation

Allosteric regulation occurs when an enzyme is bound or modified at a location other than the active site. There are two types of allosteric regulation: homotropic and heterotropic.

Homotropic Allosteric Regulation When the allosteric regulator is a substrate for the enzyme, it is called homotropic regulation. Some enzymes are comprised of multiple sub-units with multiple active sites. In many cases, these active sites interact with each other cooperatively. In other words, binding of one substrate molecule facilitates the binding of another substrate molecule at another site. Consequently, as the substrate concentration increases, the rate of the reaction increases exponentially until it becomes saturated and levels out. The resulting substrate/rate equation is a sigmoid curve, rather than a rectangular hyperbolic curve.



▲ **Figure 7–2.6F** Allosteric vs. Non-allosteric Regulation

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Heterotropic Allosteric Regulation When the allosteric regulator or modifier is not a substrate for the enzyme, it is called heterotropic regulation. Heterotropic regulators can be small molecules that bind the enzymes or covalent modifications. The most common of these modifications is phosphorylation, the addition of a phosphate group to an enzyme. This can either increase or decrease the enzyme's activity.

2.6.4 Regulation of Enzyme Number

Enzyme activity can also be regulated by increasing or decreasing the number of enzymes in the cell. The higher the number of enzymes, the more catalyzed reactions occur and vice versa. There are several mechanisms:

- Altering the rate of transcription through the action of transcription factors.
- Altering the rate of translation.
- Regulating the rate of enzyme degradation, for example, by regulating ubiquitination.

Clinical Application

Constitutive Activation of Signaling Pathways

The hallmark of cancer is unchecked cell growth. Often, this is due to mutations in genes encoding proteins responsible for promoting growth (oncogenes). Some of these mutations simply lead to higher expression of oncogenes. Others, however, alter allosteric regulatory mechanisms that normally keep growth-promoting proteins in check. For example, the Abl kinase, found on chromosome 9, is highly regulated in the cell. In chronic myelogenous leukemia (CML), the kinase becomes constitutively active through fusion with the Bcr gene on chromosome 22 to form the Bcr-Abl protein, which is no longer subject to normal allosteric regulation. It remains highly active in the cell, and leads to uncontrolled proliferation of the leukemic cells. This genetic fusion, called the Philadelphia chromosome, is found in virtually all cases of CML.

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1 Overview of Metabolic Pathways

Metabolic pathways are the interconnected networks of enzymes, substrates, and products that convert the products of food digestion to the macromolecule building blocks of our cells, tissues, and organs, and to the energy that powers them.

Each of these pathways is composed of a large number of individual enzymes, substrates, and products, the details of which could fill many textbooks. However, for the purposes of reviewing for Step 1, it is important to focus on only the most essential details. As you review each pathway, it may be helpful to ask yourself the following questions:

- **Inputs and Outputs:**
  - What is the starting material for this pathway?
  - What are the final products?
  - How does this pathway integrate with other pathways in metabolism?
- **Key Enzymatic Steps:**
  - What is the rate-limiting step of this pathway?
  - Which are the regulated steps of this pathway and how are they regulated?
  - For each rate-limiting and/or regulated step, what are the enzymes, substrates, products, and cofactors?
- **Associated Diseases:**
  - Which enzymes have known genetic defects or deficiencies that cause disease?
  - Which enzymes are implicated in other nongenetic diseases (e.g., vitamin deficiencies)?
  - What are the mechanisms, signs and symptoms, inheritance patterns, and treatments for each disease?

USMLE® Key Concepts

For Step 1, you must be able to:

- Explain regulation of metabolic pathways.
- Describe the mechanisms and effects of insulin and glucagon on metabolism.
- Identify signal transduction pathways associated with metabolic regulation.

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2 Integrated Control of Metabolism

A critical function of metabolism is maintenance of homeostasis. For example, the cells and tissues of the body need to be able to function just as well during conditions of fasting as they do after feeding. Cells need to function appropriately during exercise and at rest, during stress and calm. The processes by which homeostasis is maintained are called metabolic regulation.

There are two major types of metabolic regulation: local regulation and hormone regulation.

2.1 Local Regulation

Local regulation entails control and coordination of metabolism in a single cell, through:

- **Mass Action:** The activity of a pathway is determined by the concentration of substrates. When substrates are at high concentrations, the pathway is highly active. When the substrates are at low concentrations, the pathway is relatively inactive.
- **Feedback Regulation:** Products of a pathway regulate the activity of the enzymes that produced them. If the products inhibit the pathway, this is called negative feedback regulation; if the products stimulate the pathway, this is called positive feedback regulation.
- **Feed-Forward Regulation:** Substrates of a pathway stimulate the activity of the downstream enzymes that will metabolize them.

2.2 Hormonal Regulation

Cellular metabolism has to be coordinated in the context of the entire organism to maintain overall body homeostasis. For example, blood glucose must stay relatively constant even though carbohydrate input varies after and between meals. Hormones, substances produced by cells in one organ that regulate the biochemistry of cells in other organs, are the primary mediators of whole-body metabolic regulation.

The *insulin-glucagon axis* is one of the major hormone controls. Its purpose is to maintain energy sufficient to accommodate tissues during the feed/fast cycle. The primary hormones involved are insulin and glucagon and, in some tissues, epinephrine.

2.2.1 Glucagon and Adrenal Catecholamines

This is a polypeptide hormone produced by the *α cells of the islets of Langerhans in the pancreas*. Glucagon is secreted in response to falling blood glucose; that is, it increases in the fasted state. Its function is to make available the storage forms of energy to raise blood glucose back to the normal level. The direct effect of glucagon is primarily in the liver. In humans, hepatocytes are the major cells with glucagon receptors.

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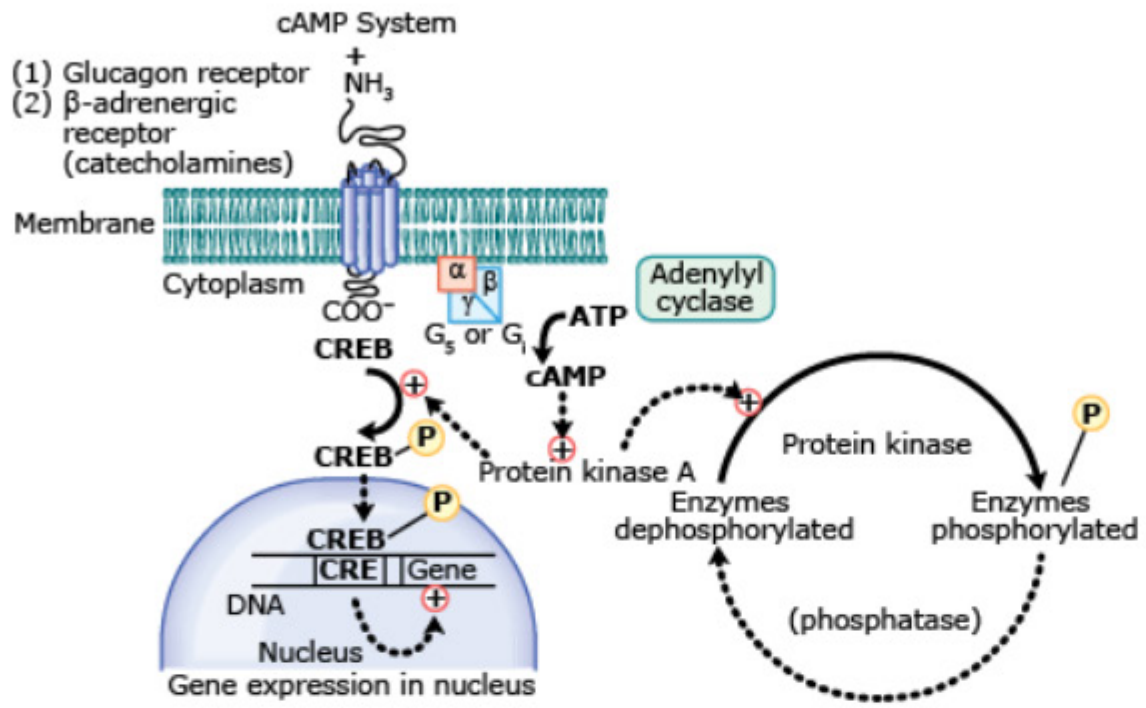
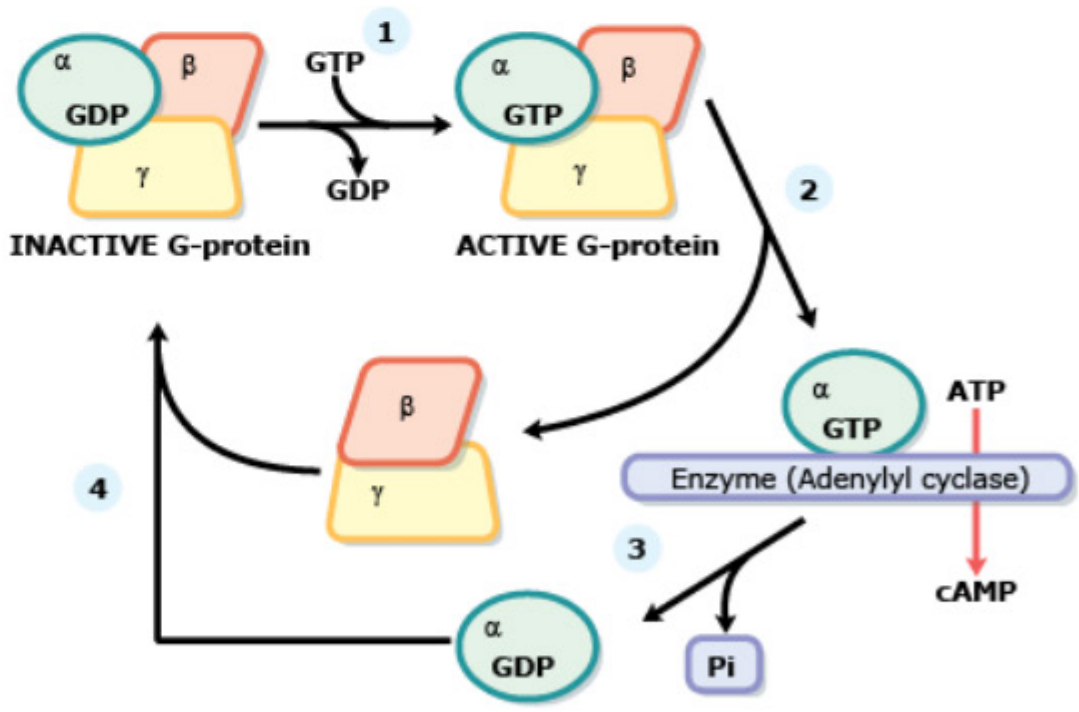
Epinephrine and *norepinephrine* are catecholamine hormones produced as part of the body's stress response. They are produced by the adrenal medulla and have effects similar to glucagon. Thus, for the purposes of this review, we will consider them together.



▲ **Figure 8–2.2A** Glucagon and Catecholamines in cAMP Signal Transduction

Glucagon functions by binding to and activating a cell surface receptor. This receptor is associated with *G protein*, a heterotrimer consisting of three subunits, α, β, and γ. When the receptor is activated, it causes the α subunit to exchange a molecule of GDP for GTP and separate from the β and γ subunits. The activated α subunit stimulates the activity of an enzyme called adenylyl cyclase, the function of which is to convert ATP to *cyclic AMP (cAMP)*.



▲ **Figure 8–2.2B** G-Protein Activation of Adenylyl Cyclase

cAMP is a second messenger, a connector between signaling pathways in the cell. It activates many things, but the most important in terms of glucagon function is *protein kinase A (PKA)*. As its name implies, PKA catalyzes phosphorylation of proteins involved in many metabolic pathways, either stimulating or inhibiting their function. In this way, it mediates the response of a cell to glucagon.

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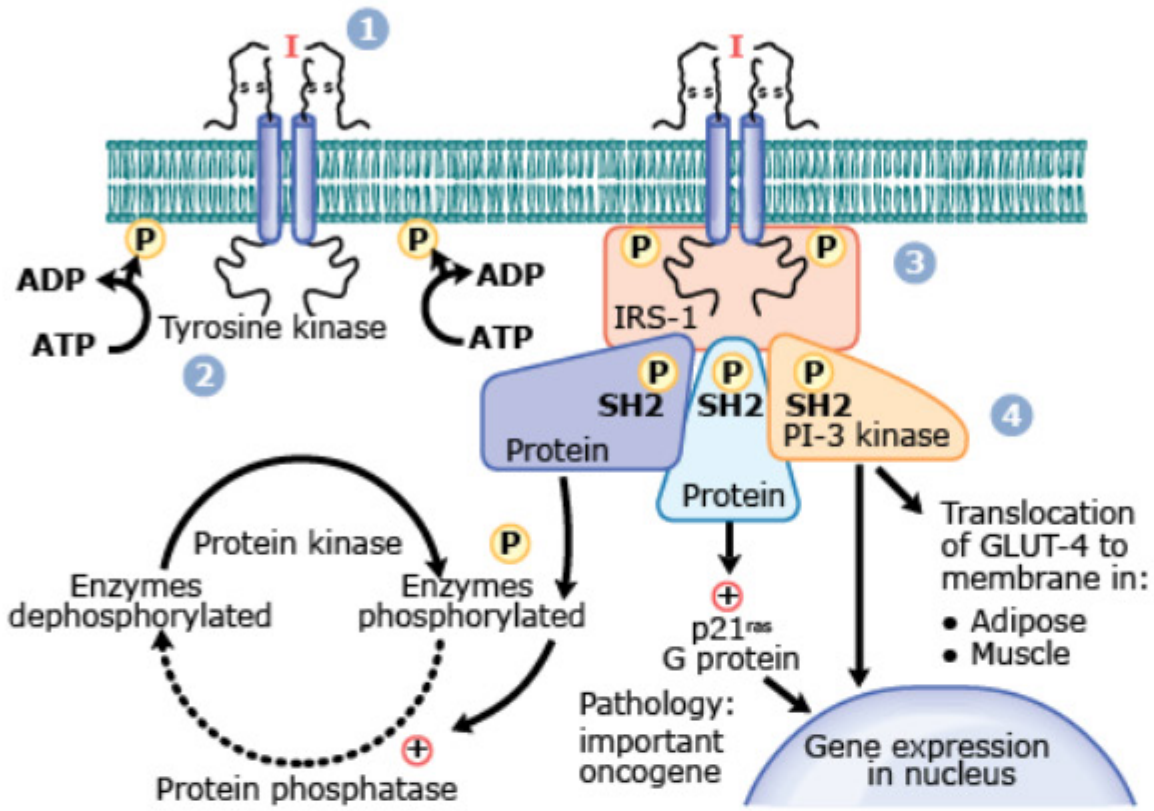
2.2.2 Insulin

This polypeptide hormone is produced by the *beta cells of the islets of Langerhans in the pancreas*. Insulin is secreted in response to rising blood glucose, usually following a meal. Its primary function is to transport glucose into cells both to increase the energy (ATP, NADH) required to convert glucose to storage forms such as glycogen or fat so they will be available during later fasting.



1 Insulin binding activates tyrosine kinase activity

2 Autophosphorylation of receptor

3 Insulin receptor substrate (IRS) binds receptor and is phosphorylated on tyrosine residues

4 SH2 domain proteins bind phosphotyrosine residues on IRS

▲ **Figure 8–2.2C** Insulin Protein Tyrosine Kinase Receptor

Insulin also binds to cell surface receptors, in this case a *receptor tyrosine kinase*. Binding of insulin causes dimerization of the receptors, such that two receptors come together and phosphorylate each other on tyrosine residues. Effector proteins in the cell bind these phosphotyrosines and transduce the signal into the cell. Similar to glucagon, insulin has many targets, but we will consider just three:

1. Insulin activates *protein phosphatases*, such as protein phosphatase 1 (PP1), which dephosphorylate enzymes and change their activity, essentially reversing the work of PKA.
2. Insulin also activates *cAMP phosphodiesterase*, which degrades cAMP to its inactive form, 5'-AMP.
3. In adipocytes and muscle cells, insulin initiates the PI-3 kinase pathway, which stimulates the translocation of vesicles with GLUT-4 to the cell membrane.

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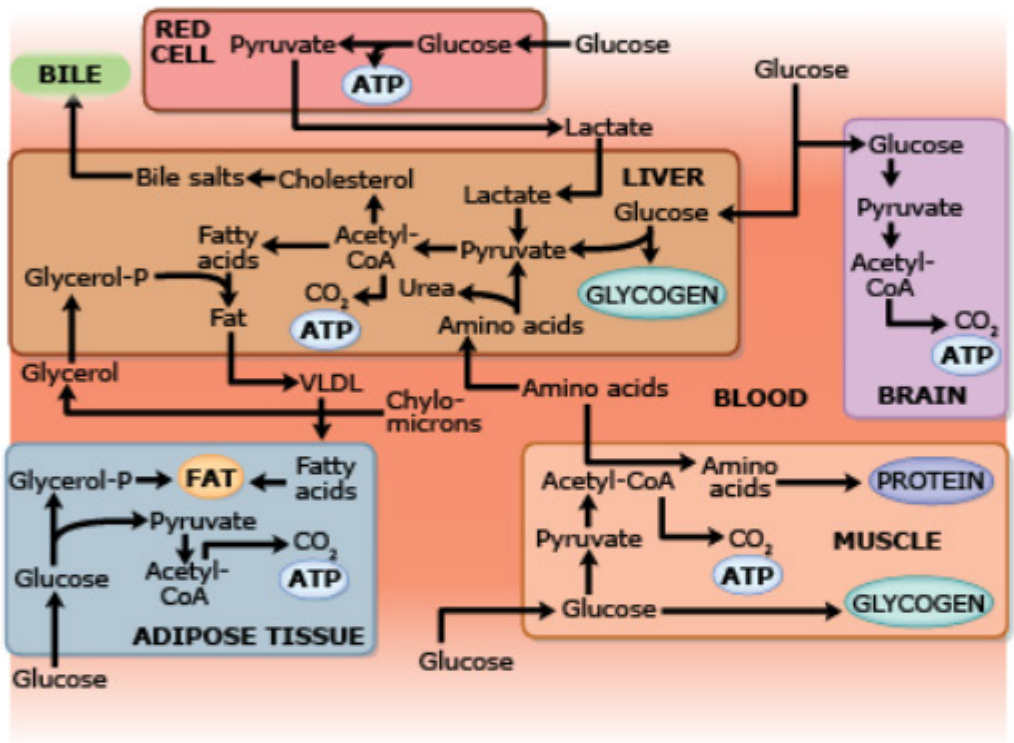
2.2.3 Glucagon vs. Insulin

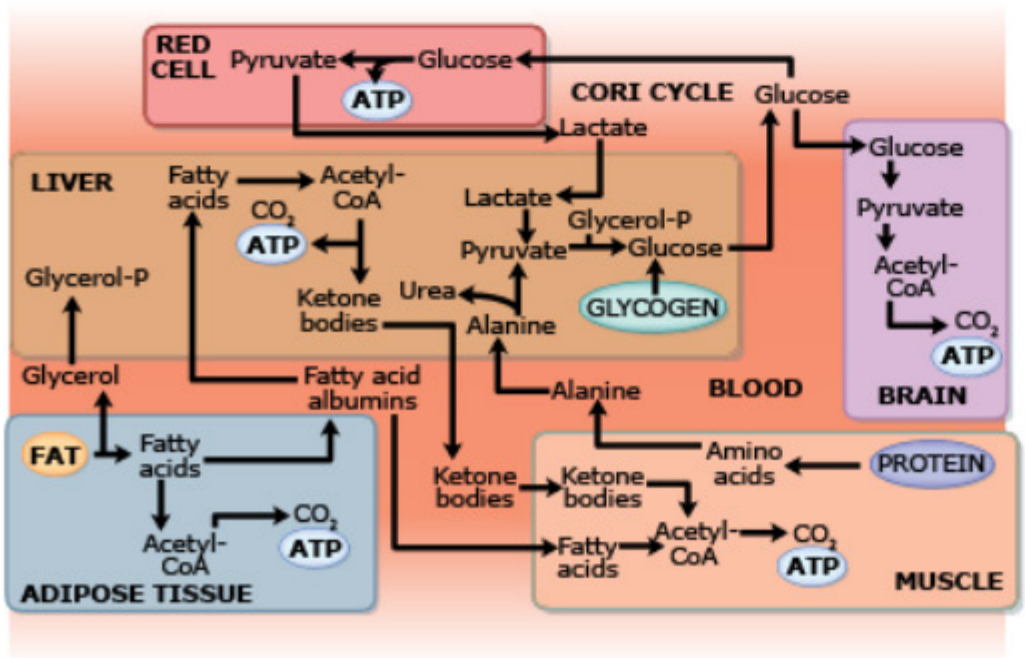
Note that the activity of glucagon is in every way the opposite of insulin as illustrated in table 8–2.2.

▼ **Table 8–2.2** Glucagon vs. Insulin

	Glucagon	Insulin
Site of Production	Alpha (α) cells	Beta (β) cells
Stimulus	Hypoglycemia (low glucose)	Hyperglycemia (high glucose)
Receptor	G-protein linked receptor	Receptor tyrosine kinase
Target	Protein kinase A (phosphorylation)	Protein phosphatases (dephosphorylation)
Effect	Releases storage forms of energy to maintain blood glucose	Stimulates glucose uptake and metabolism to storage forms



▲ **Figure 8–2.2D** Metabolic Profile of the Absorptive State



▲ **Figure 8–2.2E** Metabolic Profile of the Postabsorptive State

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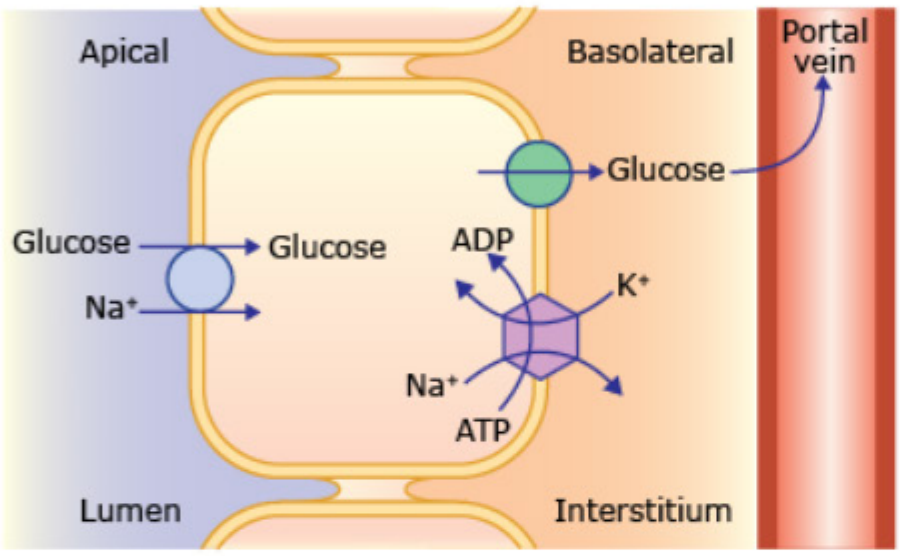
1 Glucose Absorption in the Intestine

Glucose is absorbed by intestinal epithelial cells with a molecule of Na^+ by a glucose/Na^+ symporter. This kind of cotransport is required because glucose is being transported against its concentration gradient. That is, the concentration of glucose inside the cell is higher than that outside the cell, so its import requires energy that comes from the transport of Na+ down its concentration gradient. Na^+/K^+ ATPase maintains this concentration gradient.



▲ **Figure 9–1.0** Glucose Absorption in the Intestine

From the intestinal epithelial cells, monosaccharides are transported via the portal vein to the liver, where they have several potential fates, including:

- Oxidation to CO_2 and water with the resulting production of energy.
- Storage as glycogen.
- Metabolism to fatty acids for storage in adipose tissue.
- Release to circulation for metabolism by other tissues.

Normal fasting blood glucose is <100 mg/dL. However, levels below 40–50 mg/dL for males or 35–40 mg/dL for females are termed *hypoglycemia*. These levels are accompanied by adverse symptoms, such as fatigue, lethargy, headache, dizziness, confusion, and stupor. On the opposite side is *hyperglycemia*, which could be a sign of diabetes mellitus, a syndrome of impaired glucose metabolism. Fasting glucose of 100–125 mg/dL indicates impaired glucose tolerance, with levels ≥126 mg/dL diagnostic of diabetes.

▼ **Table 9–1.0** Clinical Fasting Serum Glucose Standards

Condition	Fasting Serum Glucose
Hypoglycemia	<45–50 mg/dL (males) <35–40 mg/dL (females)
Normal	<100 mg/dL
Impaired glucose tolerance	100–125 mg/dL
Diabetes mellitus	>126 mg/dL

USMLE® Key Concepts

For Step 1, you must be able to:

- Explain how dietary glucose reaches peripheral tissues.
- Identify the key steps of glycolysis.
- Describe the regulation of glycolysis.
- Explain how fructose and galatose enter glycolysis.

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2 Glucose Uptake

Glycolysis begins with glucose transport into cells by facilitated diffusion using a family of transporters (GLUT).

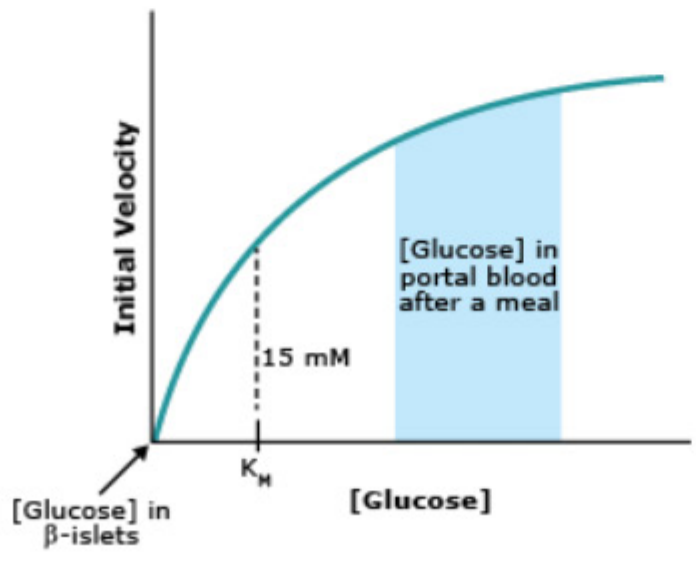
▼ **Table 9–2.0** Membrane Glucose Transport Proteins

Transporter	Tissues	K_M for Glucose*	Function
GLUT-1	• Erythrocytes • Brain • Most tissues	1 mM	Basal glucose uptake
GLUT-2	Hepatocytes	15 mM	Uptake and release of glucose
	β-islet cells of pancreas		Glucose sensor
GLUT-3	• Erythrocytes • Brain • Most tissues	1 mM	Basal glucose uptake
GLUT-4	Adipose	5 mM	Insulin-sensitive glucose uptake
	Muscle		Insulin- and exercise-stimulated

*Normal blood glucose concentration is 4 – 6 mM (72 – 110 mg/dL).

2.1 GLUT-2 Function in Hepatocytes

The primary function of glucose entering the liver in the absorptive phase is to produce storage forms of glucose (glycogen and fatty acids). Hepatocytes also oxidize a much smaller amount of incoming glucose to increase available ATP to support these storage pathways. However, GLUT-2 has a higher K_M for glucose (lower affinity) than all the other transporters listed in Table 9–2.0. This seems rather paradoxical; why should the liver, which is primarily converting portal blood glucose to storage forms, have such a low affinity for glucose? The answer to this question lies in imagining what would happen if the hepatocytes had a low K_M, high affinity transporter. The liver would then remove so much glucose that there would be little left for the peripheral tissues. After a meal, the glucose concentration in the *portal blood can reach 10–15 mM,* in contrast to the arterial blood that has a glucose concentration closer to 5 mM. Therefore, there is sufficient glucose in the portal blood to drive GLUT-2 and leave enough glucose in the blood for peripheral tissues.



▲ **Figure 9–2.1** GLUT-2 Kinetics in Liver and β-Islet Cells of the Pancreas

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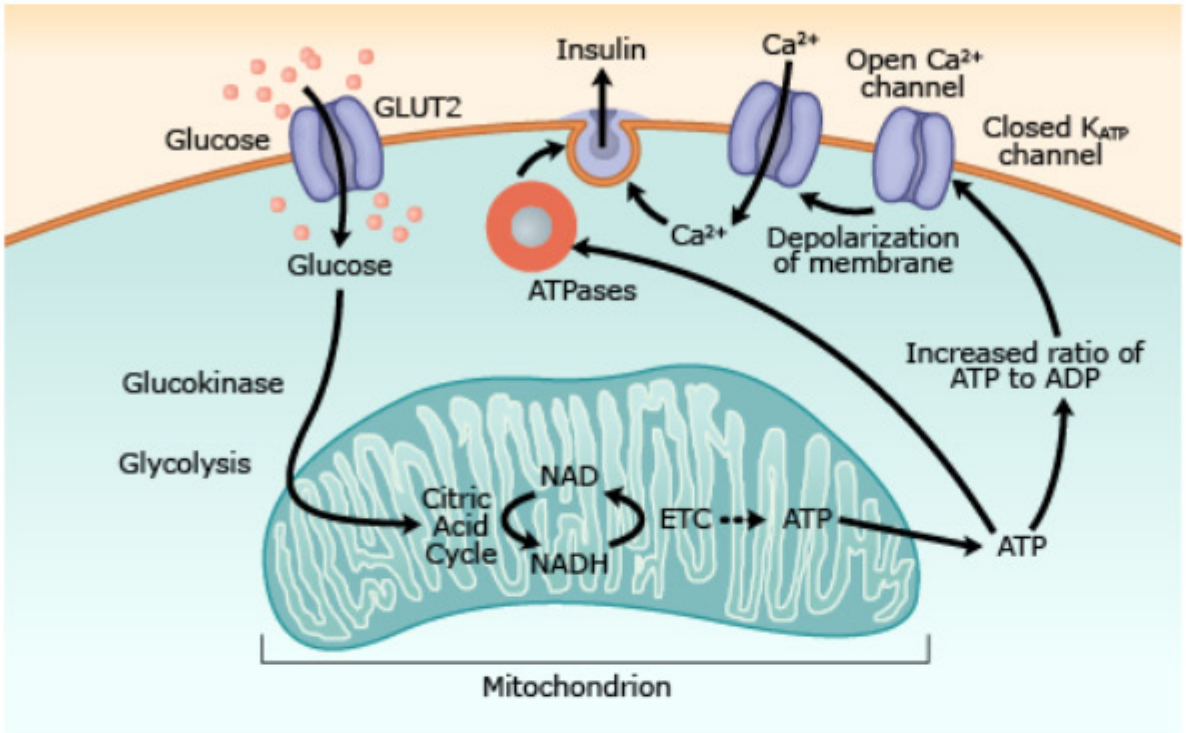
2.2 GLUT-2 Function in Pancreatic β-islet Cells

Unlike the hepatocytes, the pancreatic β-islet cells are exposed to glucose in the peripheral blood where the glucose concentration is closer to 5 or 6 mM. This places the glucose concentration beneath the K_M for glucose, which is 15 mM. In this case, the rate of glucose transport is much more responsive to the concentration of glucose in the peripheral blood (see Figure 9–2.2).



▲ **Figure 9–2.2** Glucose-Stimulated Insulin Release

2.3 GLUT-4 in Adipose and Muscle

GLUT-4 activity is increased in adipose and muscle tissue by insulin. The mechanism does not involve gene expression and is therefore rapid. Within both cell types, the increase is due to insulin stimulating the translocation of vesicles, whose membranes are densely populated by GLUT-4 transporters, to the cell membrane in both muscle and adipose.

The resulting influx of extra glucose in the adipocyte provides the precursor to DHAP and glycerol-3-P required to store triglycerides in adipose tissue.

The extra GLUT-4 in muscle tissue allows a more rapid uptake of glucose to replace muscle glycogen depleted during activity. Muscle also is the major tissue to move glucose out of the blood stream, thus decreasing glucose concentration in the periphery in the absorptive state. An additional aspect of GLUT-4 in the muscle is that its translocation to the cell membrane can also be stimulated by exercise—independently of insulin.

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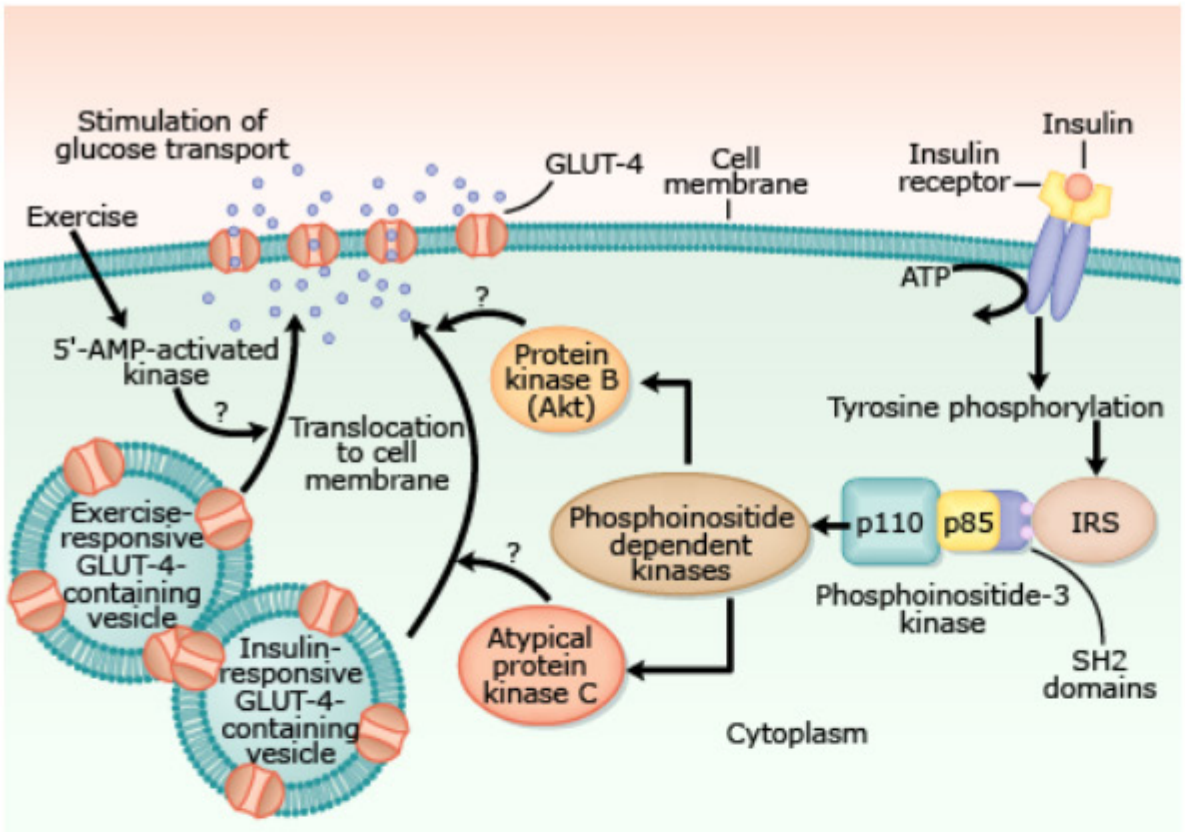

▲ **Figure 9–2.3** Stimulation of Glucose Transport in Skeletal Muscle by Insulin and Exercise

That GLUT-4 activity can be increased by both insulin and exercise is an especially important consideration for diabetics. The effects of exercise are summarized in Table 9–2.3.

▼ **Table 9–2.3** Effects of Exercise on Tissue Glucose Levels

Individual	Glucose Uptake	Blood Glucose	Blood Insulin	Result
Non-diabetic	Increases	Decreases	Decreases	Normoglycemia
Type 1 diabetic	Increases	Decreases	Little change	Hypoglycemia
Type 2 diabetic	Increases	Decreases	Depends on degree of insulin resistance	Normoglycemia

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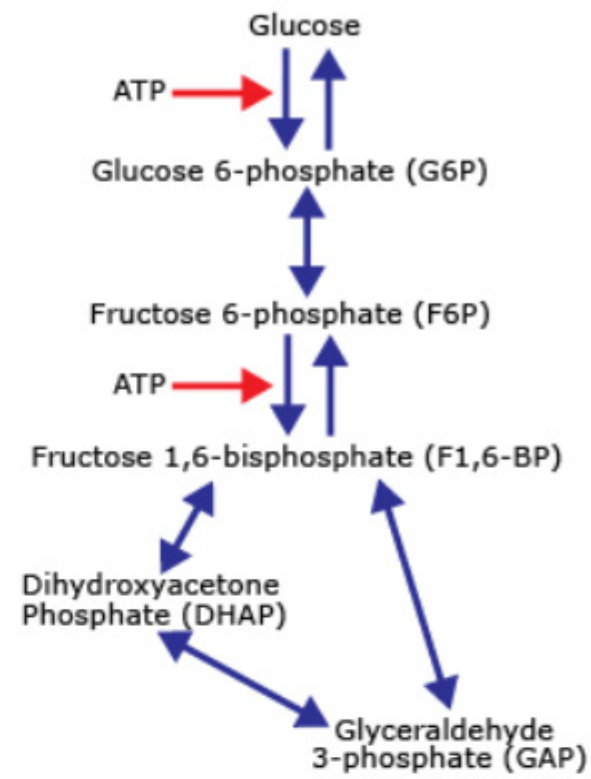
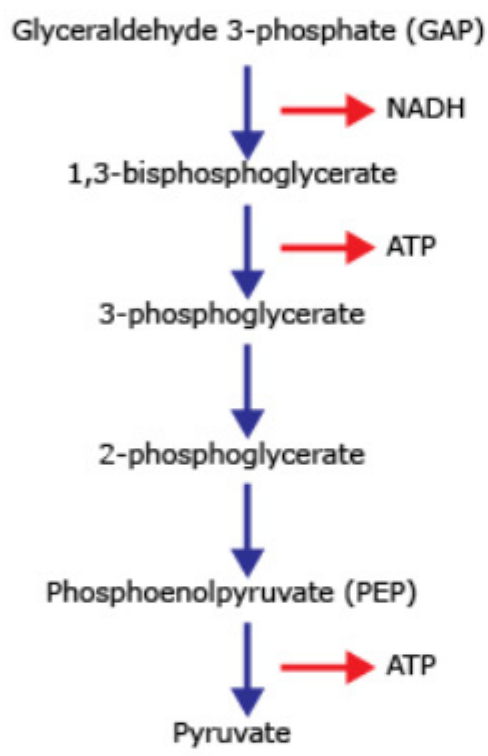
3 Glycolysis

In glycolysis, one molecule of glucose is converted to two molecules of pyruvate. This occurs in two phases:

1. 6-carbon glucose is converted to two 3-carbon glyceraldehyde 3-phosphates in five enzymatic steps. These are energy-consuming reactions, requiring two molecules of ATP.
2. Glyceraldehyde 3-phosphate is converted to pyruvate in five enzymatic steps. These are energy-producing steps, generating two molecules of ATP and one molecule of NADH for each glyceraldehyde 3-phosphate. Thus, four ATP and two NADH are produced for each molecule of glucose converted to pyruvate.



▲ **Figure 9–3.0A** Glycolysis: Glucose to Glyceraldehyde 3-Phosphate



▶ **Figure 9–3.0B** Glycolysis: Glyceraldehyde 3-Phosphate to Pyruvate

The net reaction for glycolysis in aerobic cells is:

$$1 \text{ glucose} + 2 \text{ ADP} + 2 \text{ P}_i + 2 \text{ NAD}^+ \rightarrow 2 \text{ pyruvate} + 2 \text{ ATP} + 2 \text{ NADH}$$

Of the 10 enzyme catalyzed steps of glycolysis, seven are reversible (have a sufficiently small ΔG), and thus these reactions can go in either direction, depending on the concentration of substrates and products for the reactions. These reactions are catalyzed by the same enzyme in both directions.

There are *three steps* that have a sufficiently large, negative ΔG such that they are essentially irreversible in the cell. These reactions can be used to convert glucose to pyruvate, but under normal conditions, cannot be used to convert pyruvate to glucose. These are the rate-limiting steps and the primary targets of local and hormonal regulation. As such, these reactions, and the enzymes that catalyze them, are *high yield for Step 1*.

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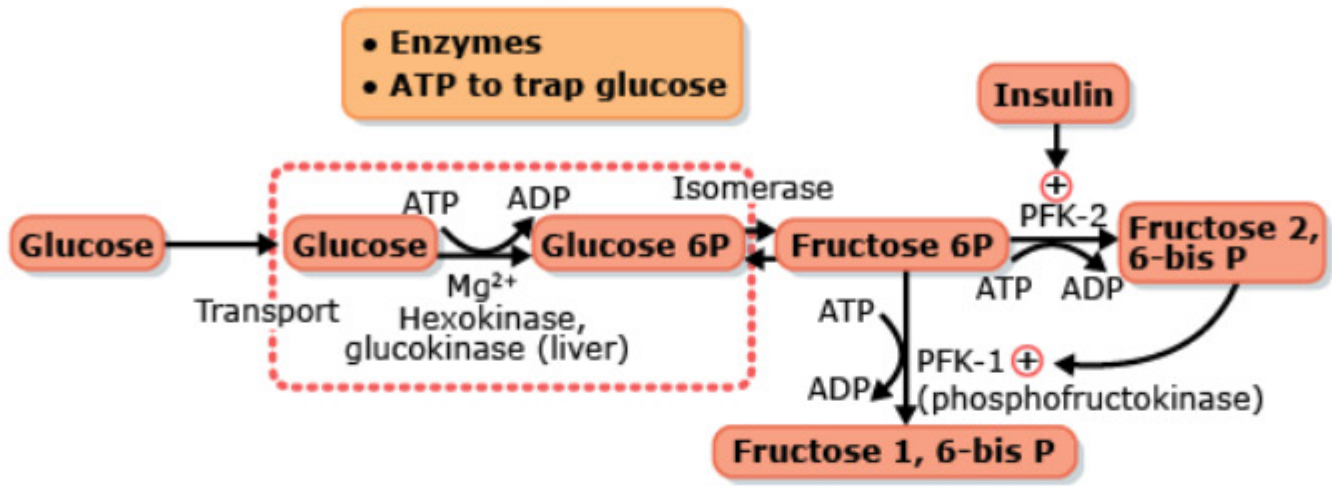
3.1 Step One: Glucose + ATP → Glucose 6-Phosphate (G6P) + ADP

The GLUT transporters move glucose in both directions. So in order to retain glucose in the cell, it must first be phosphorylated. Glucose 6-phosphate (G6P) is not a substrate for these transport proteins and the reaction shown in Figure 9–3.1A is irreversible under cell conditions.



Hexokinase	Glucokinase
Most tissues	Hepatocytes and pancreatic cells β-islet
Low K_M (0.05 mM in erythrocytes)	High K_M (10 mM)
Inhibited by glucose 6-phosphate	Induced by insulin in hepatocytes

▲ **Figure 9–3.1A** Roles of Hexokinase and Glucokinase

This reaction can be catalyzed by one of two enzymes, *hexokinase* and *glucokinase*.

Hexokinase is expressed in all tissues and it is has lower specificity than glucokinase—it can also phosphorylate fructose and galactose. It has a low K_M, such that it functions maximally at low blood glucose levels. Thus, similar to GLUT-1 and -3, it functions in basal glucose metabolism. Hexokinase is feedback inhibited, however, by high concentrations of G6P, so that cells do not use more glucose than is needed.

Glucokinase is found in the liver and pancreatic beta cells. Unlike hexokinase, it has high specificity for glucose and has a comparatively high K_M. This is similar to the GLUT-2 transporter that is expressed in the same tissues in that both function primarily when blood glucose levels are high, such as after a meal. Unlike hexokinase, glucokinase is not feedback inhibited by G6P. In coordination with GLUT-2, this allows for the accumulation and metabolism of excess glucose by pancreatic beta cells, which secrete insulin in response, and by the liver, which converts glucose to storage forms such as glycogen and fatty acids.

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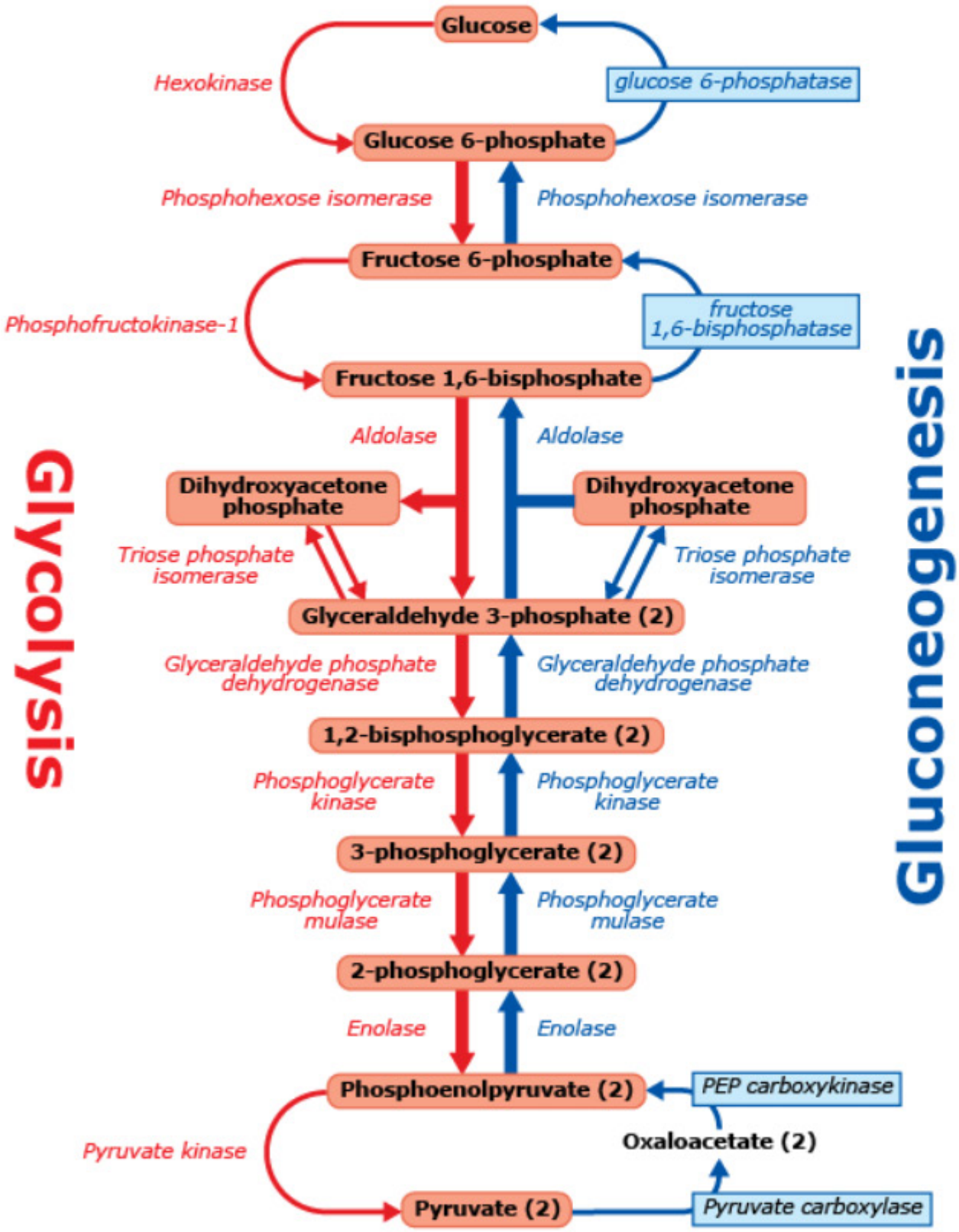
In gluconeogenesis, G6P is dephosphorylated back to glucose. This reaction is catalyzed by *glucose-6-phosphatase*. Unlike the other enzymes of glycolysis and gluconeogenesis that are found in the cytoplasm, this enzyme is in the lumen of the endoplasmic reticulum. Thus, a transporter is required to bring G6P into the endoplasmic reticulum and move glucose and inorganic phosphate back out to the cytoplasm. This enzyme is not found in the brain or the muscle, so gluconeogenesis cannot occur in these tissues. Rather, this enzyme is expressed most highly in the liver, the major organ for gluconeogenesis.



▲ **Figure 9–3.1B** Comparison of Glycolysis and Gluconeogenesis Pathways

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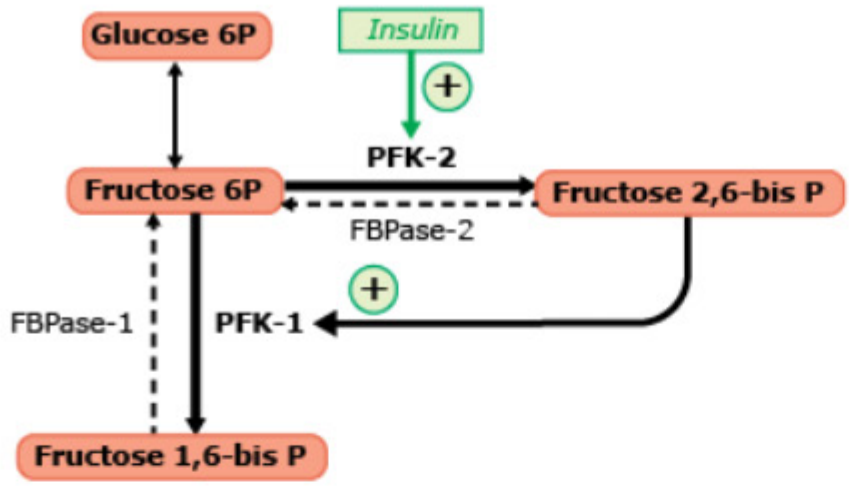
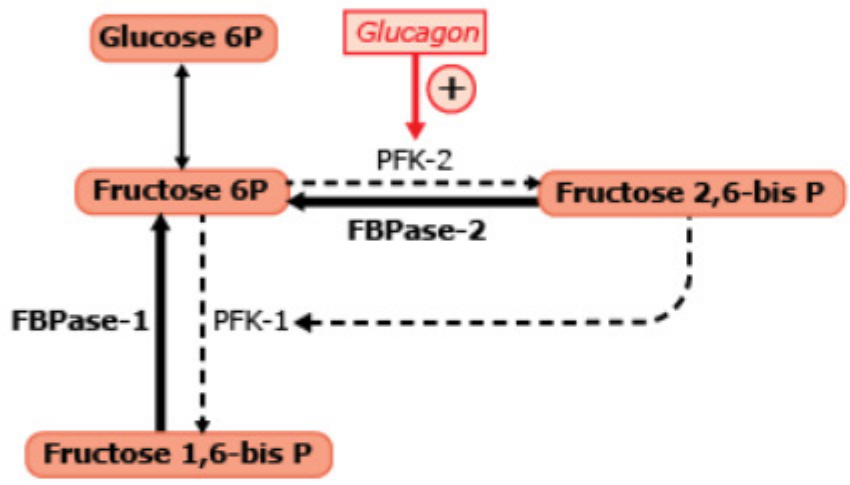
3.2 Step Two: Fructose 6-Phosphate → Fructose 1,6-Bisphosphate



▲ **Figure 9–3.2A** Glycolysis: Fructose 6-Phosphate to Fructose 1,6-Bisphosphate (Forward Reaction)



▲ **Figure 9–3.2B** Gluconeogenesis: Fructose 1,6-Biphosphate to Fructose 6-Phosphate (Reverse Reaction)

The forward reaction in this step is catalyzed by *phosphofructokinase-1 (PFK-1)*. The reverse reaction (in gluconeogenesis) is catalyzed by *fructose 1,6-bisphosphatase-1 (FBPase-1)*. These are the primary rate-limiting reactions of glycolysis and gluconeogenesis. Thus, they are the most important steps for regulation of glucose metabolism.

At the local or cellular level, this reaction is regulated by an energy balance between higher energy ATP and lower energy ADP and AMP. ATP is a negative regulator of PFK-1, as is citrate. AMP is a positive regulator of this enzyme.

PFK-1 reactions are also regulated hormonally by insulin and glucagon. The key element of hormonal regulation is another derivative of F6P, called *fructose 2,6-bisphosphate (F2,6-BP)*, which is an allosteric regulator of PFK-1. F2,6-BP activates PFK-1 by increasing its affinity for F6P and decreasing the inhibitory effect of ATP. Thus, hormonal control can at least partially override the energy control of PFK-1.

Important Concept

- PFK-1 is the primary regulatory point for glycolysis.
- FBPase-1 is the primary regulatory point for gluconeogenesis.

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Remember that the primary function of glucagon is activation of kinases, such as protein kinase A (PKA), leading to protein phosphorylation. Thus, in the fasting state, blood glucose falls, stimulating glucagon secretion. One result is the phosphorylation of the bifunctional enzyme. Under these conditions, PFK-2 activity is decreased and FBPase-2 activity is increased, resulting in lower concentrations of F2,6-BP. When F2,6-BP levels are low, PFK-1 is inhibited, decreasing glycolysis, and FBPase-1 is stimulated, turning on gluconeogenesis. This is the appropriate response to hypoglycemia—a shift from glucose consumption (glycolysis) to glucose production (gluconeogenesis).

Conversely, one of the primary functions of insulin is activation of phosphatases, leading to dephosphorylation of proteins. Thus, in the fed state, blood glucose rises, stimulating insulin secretion. One result is the dephosphorylation of the bifunctional enzyme. Under these conditions, PFK-2 activity is increased and FBPase-2 activity is decreased, resulting in higher concentrations of F2,6-BP. When F2,6-BP levels are high, PFK-1 is stimulated, turning on glycolysis, and FBPase-1 is inhibited, turning off gluconeogenesis. This is the appropriate response to hyperglycemia—a shift from glucose production (gluconeogenesis) to glucose consumption (glycolysis).

3.3 Step Three: Phosphoenolpyruvate (PEP) → Pyruvate

In glycolysis, this reaction is catalyzed by pyruvate kinase, which generates a molecule of ATP for each of the PEP molecules it metabolizes. This enzyme is regulated in several ways:

- **Positive Feed-Forward Regulation:** Fructose 1,6-bisphosphate, the product of PFK-1, stimulates pyruvate kinase activity. This allows coordinated activity between the crucial steps of glycolysis.
- **Negative Feedback Regulation:** ATP inhibits pyruvate kinase, slowing glycolysis in the setting of positive energy balance. Alanine, an amino acid that can contribute to gluconeogenesis in the fasting state, also inhibits pyruvate kinase, helping to slow glycolysis during fasting.
- **Hormonal Regulation:** Glucagon stimulates the phosphorylation and inactivation of pyruvate kinase, while insulin causes dephosphorylation and activation.

The reverse reaction in gluconeogenesis requires two enzymes with an intermediate (oxaloacetate) that is shared by gluconeogenesis and the TCA cycle. Pyruvate is converted to oxaloacetate by *pyruvate carboxylase*. This requires energy from a molecule of ATP. Oxaloacetate is then metabolized to PEP by *PEP carboxykinase (PEPCK)*. This requires energy from a molecule of GTP.

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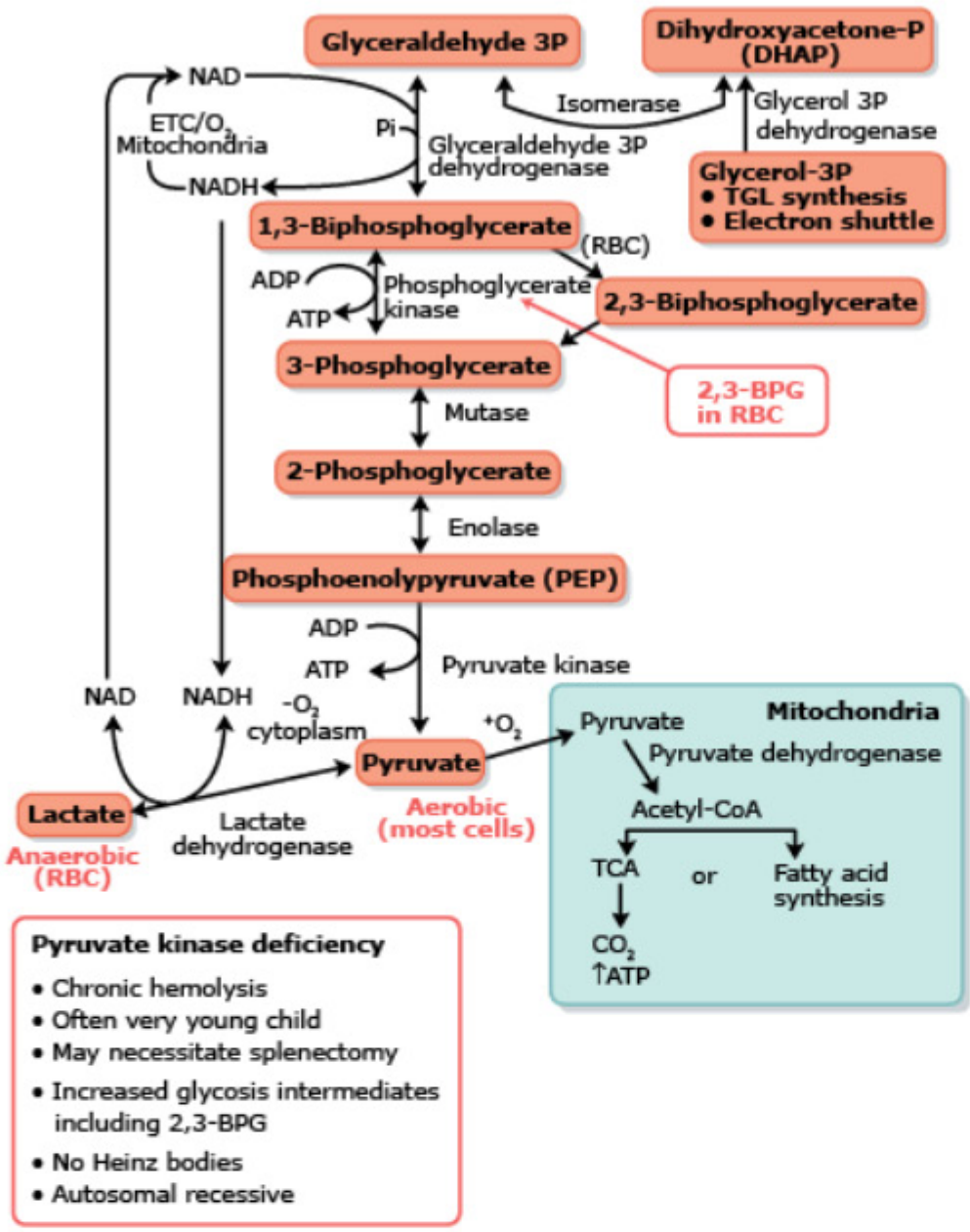

▲ **Figure 9–3.3** Pyruvate Kinase Regulation

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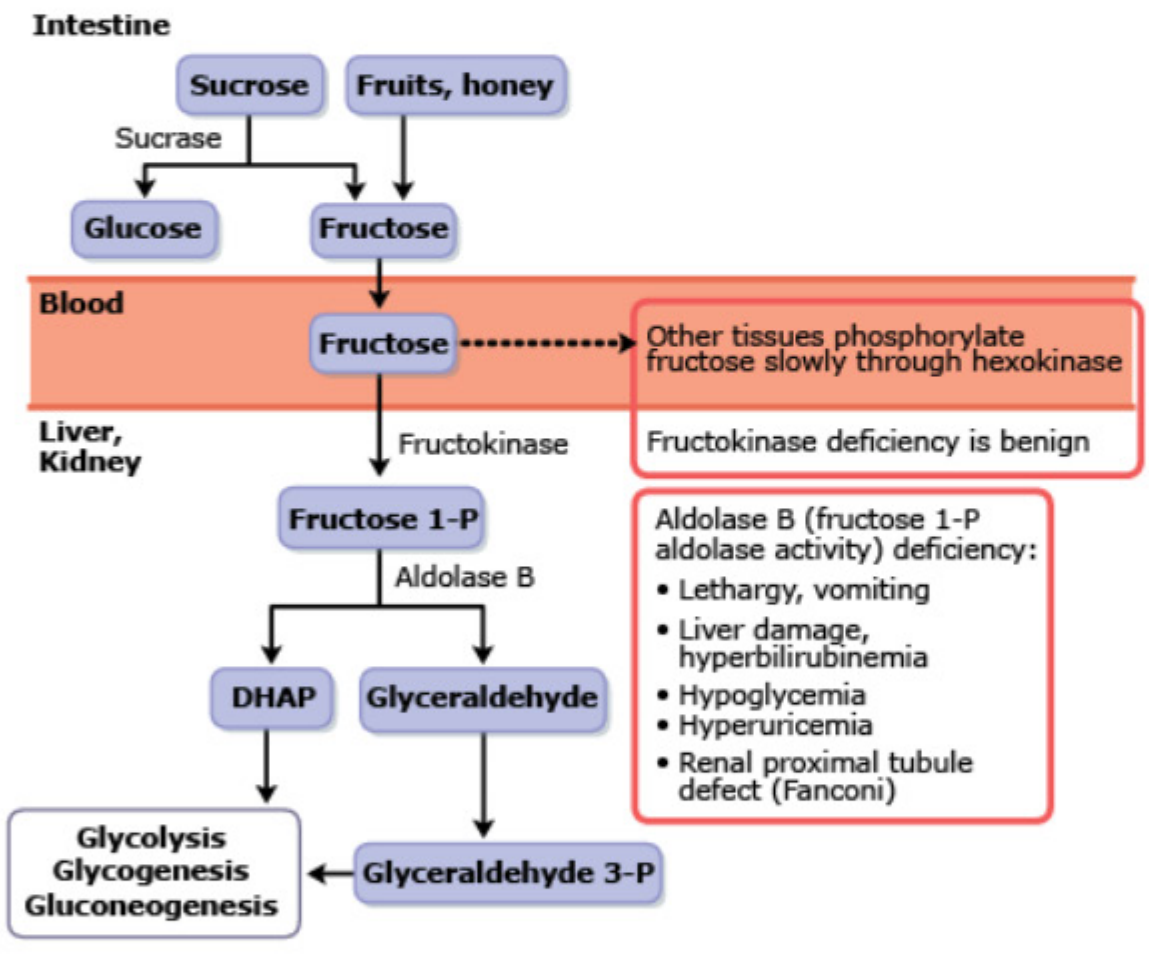
4 Fructose Metabolism

Fructose is a component of the disaccharide sucrose (table sugar). It is also found as a monosaccharide in many foods, including fruit and honey. Fructose is metabolized by conversion to intermediates of glycolysis. There are two primary mechanisms.

The most common mechanism occurs primarily in the liver. Fructose is phosphorylated to fructose 1-phosphate by *fructokinase*. Fructose 1-phosphate is then split to form dihydroxyacetone phosphate and glyceraldehyde (which is phosphorylated to glyceraldehyde 3-phosphate). This reaction is catalyzed by *aldolase B* (fructose 1-P aldolase).

The second mechanism of fructose metabolism occurs in other tissues. At high concentrations, fructose can be phosphorylated directly to fructose 6-phosphate, another intermediate of glycolysis, by *hexokinase*.



▲ **Figure 9–4.0** Fructose Metabolism

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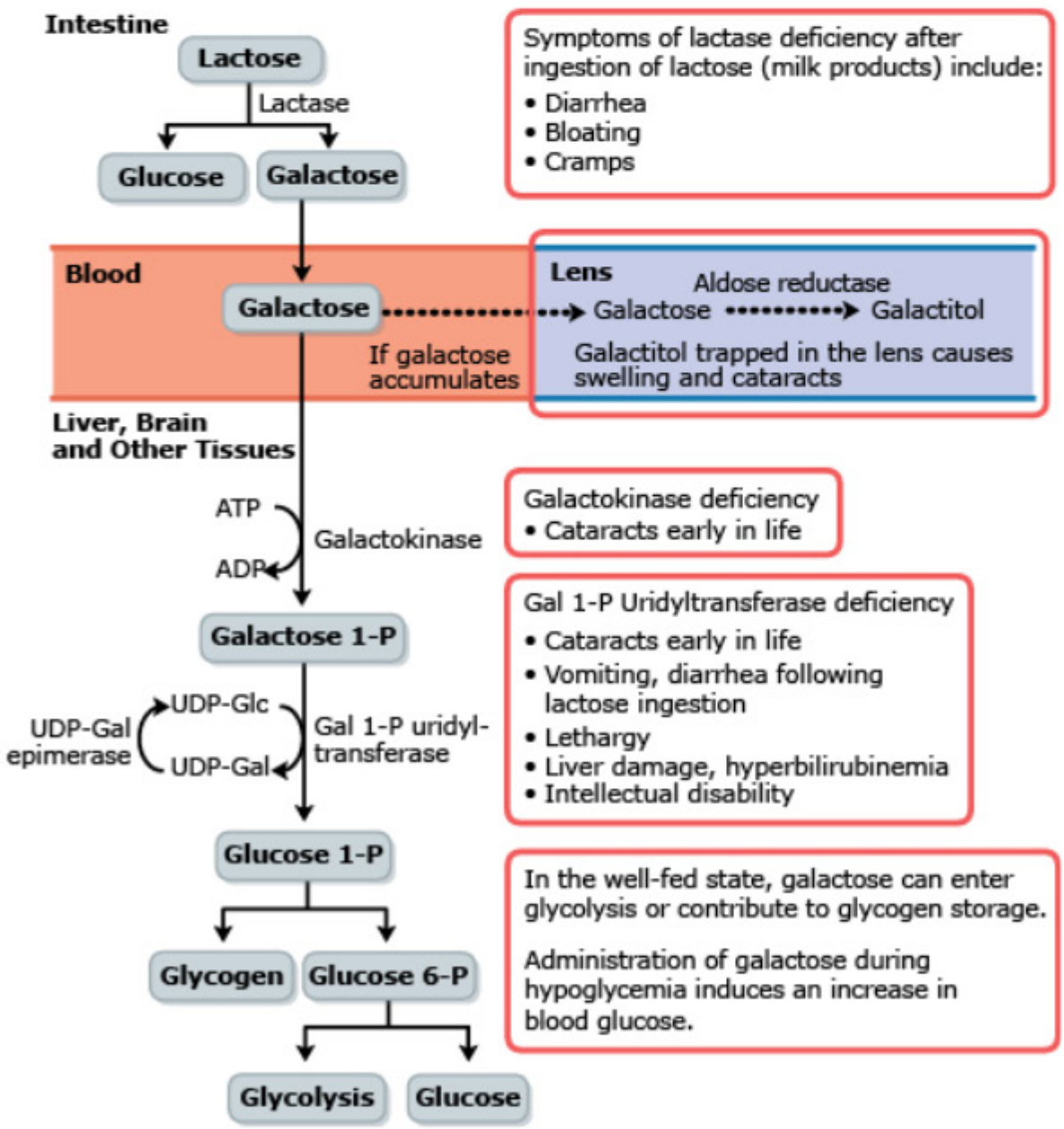
5 Galactose Metabolism

Galactose is primarily found as a component of the disaccharide lactose (milk sugar). It enters glycolysis by being converted to glucose 6-phosphate in four steps:

1. Galactose is phosphorylated to galactose 1-phosphate.
2. There is an exchange reaction between galactose 1-phosphate and UDP-glucose (a molecule of glucose covalently bonded to the nucleotide UDP), resulting in glucose 1-phosphate and UDP-galactose.
3. The glucose 1-phosphate is converted to glucose 6-phosphate that then enters glycolysis.
4. The UDP-galactose is converted to UDP-glucose by epimerase.

The net result is conversion of galactose to glucose 6-phosphate. The key enzyme in this pathway is called *galactose 1-phosphate uridyl transferase or GALT*. GALT is the enzyme responsible for catalyzing the exchange reaction between galactose 1-phosphate and UDP-glucose.



▲ **Figure 9–5.0** Galactose Metabolism

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CHAPTER 10 TCA Cycle and Oxidative Phosphorylation

1 Pyruvate and the TCA Cycle

There are three potential fates for the pyruvate produced by glycolysis:

1. Gluconeogenesis, which will be discussed in chapter 9.
2. Oxidation in the tricarboxylic acid (TCA) cycle to produce reducing equivalents that are used in oxidative phosphorylation.
3. Conversion to lactic acid.
4. Conversion to alanine.

1.1 Metabolism of Pyruvate

In the absence of gluconeogenesis, pyruvate is converted either to acetyl-CoA (for oxidation in the TCA cycle) or to lactic acid. Which pathway is followed depends on the presence or absence of oxygen.

1.1.1 Pyruvate Dehydrogenase Reaction

Pyruvate dehydrogenase catalyzes the metabolism of pyruvate to acetyl-CoA, removing one carbon in the form of CO_2. As with most enzymes that contain "dehydrogenase" in their name, it catalyzes an oxidation-reduction reaction. Pyruvate is oxidized to acetyl-CoA and, in the process, NAD^+ is reduced to NADH. This is an irreversible reaction—acetyl-CoA cannot be converted back to pyruvate. Thus, it is not a substrate for gluconeogenesis.

This reaction occurs in the mitochondrial matrix and so requires the import of pyruvate across the inner mitochondrial membrane. This is catalyzed by an ATP-dependent pyruvate transporter.

Pyruvate dehydrogenase is a complex of three different protein subunits, E1, E2, and E3, each of which utilizes a different cofactor:

- E1: Thiamine pyrophosphate (from vitamin B1)
- E2: Lipoic acid
- E3: Flavin adenine dinucleotide (FAD; from vitamin B2)

Under aerobic conditions, NADH is readily consumed by oxidative phosphorylation. Thus, the NAD^+/NADH ratio is high, which drives this reaction forward due to high substrate concentrations. The reaction is feedback inhibited by NADH, ATP, and acetyl-CoA. This regulation is mediated through phosphorylation of the E1 subunit by pyruvate dehydrogenase kinase.

1.1.2 Lactate Dehydrogenase Reaction

Under anaerobic conditions, such as in red blood cells or during hypoxic stress, NADH (generated in glycolysis, the TCA cycles, and by pyruvate dehydrogenase) builds up because it cannot be used by oxidative phosphorylation in the absence of oxygen. This inhibits pyruvate dehydrogenase and pushes pyruvate down another pathway that consumes the excess NADH.

USMLE® Key Concepts

For Step 1, you must be able to:

- Describe two of the three fates of pyruvate produced by glycolysis.
- Explain the TCA, and its inputs and outputs.
- Identify the regulatory points of the TCA cycle.
- Describe the process of oxidative phosphorylation, the end point of glucose metabolism.

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Lactate dehydrogenase catalyzes the conversion of pyruvate to lactate. This is a reduction reaction that is driven by oxidation of NADH to NAD^+. This is a critical reaction because it prevents a build-up of NADH that would otherwise inhibit glycolysis, the only source of ATP under hypoxic conditions.

Excess lactate can also be dangerous, as it causes acidosis. To prevent this, circulating lactate is taken up by hepatocytes, wherein it is converted back to pyruvate. This is also catalyzed by lactate dehydrogenase and generates a molecule of reduced NADH. This reverse reaction is favored because concentrations of lactate are much higher than pyruvate, which is rapidly metabolized, either to acetyl-CoA (by pyruvate dehydrogenase) or glucose (by gluconeogenesis), depending on the conditions.

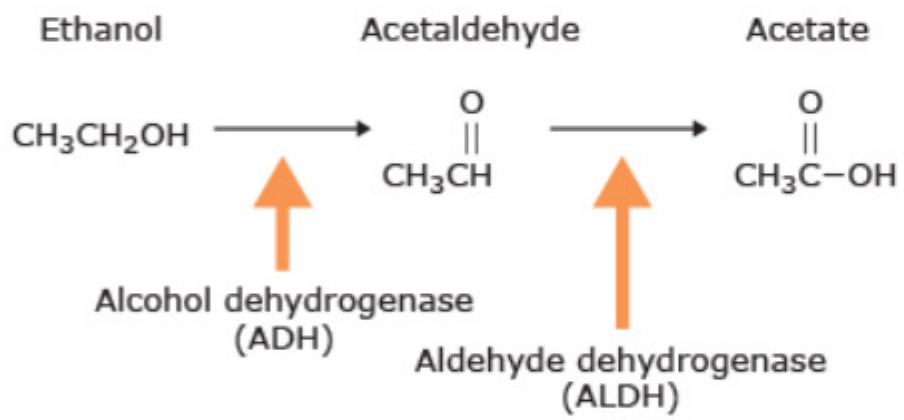
This cycle (glucose → pyruvate → lactate in tissues; lactate → pyruvate → glucose in the liver) is called the *Cori cycle*.

1.2 Metabolism of Alcohol

Another source of acetyl-CoA are alcohols, the most common of which is *ethanol* (CH_3CH_2OH). There are two major sources of ethanol:

- Dietary, primarily from alcoholic beverages
- Fermentation by gut bacteria

Ethanol is metabolized to acetate via an acetaldehyde intermediate.



▲ **Figure 10–1.2A** Ethanol Metabolism

This conversion is catalyzed by *alcohol dehydrogenase (ADH)*, which oxidizes ethanol to acetaldehyde. Acetaldehyde is further oxidized to acetate by *aldehyde dehydrogenase (ALDH)*. Both enzymes produce a molecule of reduced NADH and both require thiamine pyrophosphate as a cofactor.

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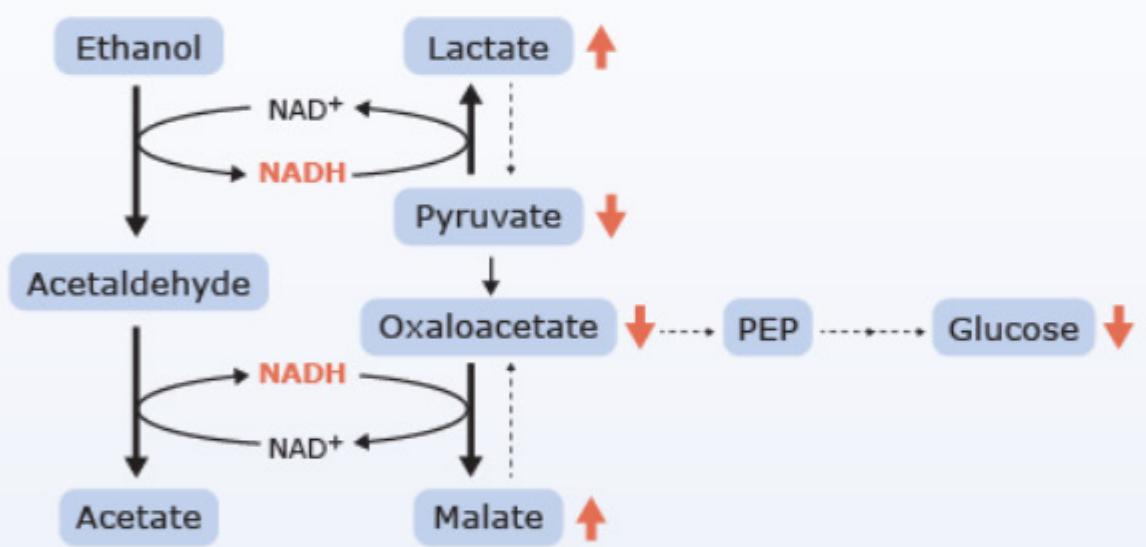
Clinical Application

Alcoholic Hypoglycemia

Hypoglycemia is a known complication of chronic alcohol abuse. The mechanism has to do with the metabolism of ethanol. Because both alcohol dehydrogenase and aldehyde dehydrogenase produce NADH, ethanol consumption lowers the NAD^+/NADH ratio. Among the consequences of this change are shifts in reversible redox reactions that involve NADH. In particular, it shifts the lactate dehydrogenase reaction towards lactate production from pyruvate and the malate dehydrogenase reaction to malate production from oxaloacetate. This reduces the levels of both pyruvate and oxaloacetate, key intermediates in the first step of gluconeogenesis. In malnourished individuals with low glycogen stores, this may result in profound hypoglycemia.



▲ **Figure 10–1.2B** Alcoholic Hypoglycemia

All of these dehydrogenase reactions require thiamine, which also may be deficient in chronic alcohol abusers. Correction of hypoglycemia with parenteral glucose, which further increases thiamine demands, can precipitate acute thiamine deficiency, known as Wernicke-Korsakoff syndrome. Thus, thiamine should always be given before glucose to correct alcoholic hypoglycemia.

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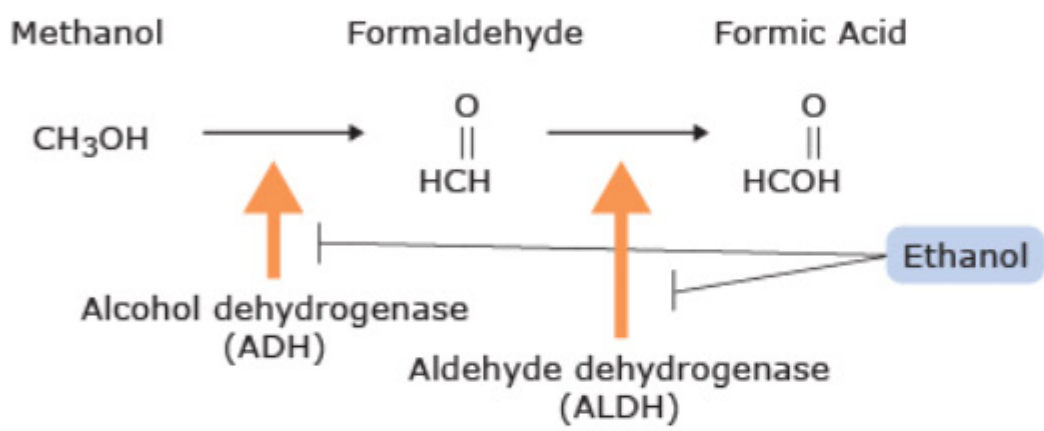
Clinical Application

Methanol Poisoning

Methanol and ethanol are metabolized by the same pathway, but the products of methanol metabolism are formaldehyde and formic acid, which are toxic. In particular, they damage the optic nerve, leading to blindness. Among the treatments is ethanol administration, which competes with the methanol for alcohol dehydrogenase, slowing the production of these toxic products.



▲ **Figure 10–1.2C** Ethanol Inhibition of Methanol Metabolism

1.3 TCA Cycle

Acetyl-CoA, produced from pyruvate or ethanol, has four possible fates. The most common fate is oxidation in the TCA cycle. The other three (fatty acid, ketone body, and amino acid production) will be discussed later.

The TCA cycle has different names. TCA, or tricarboxylic acid cycle, refers to the carboxyl group-containing intermediates of the cycle. It is also known as the citric acid cycle, after the first intermediate, or the Krebs cycle, after the biochemist who first described it.

1.3.1 Inputs and Outputs

The most basic view of the TCA cycle is that it oxidizes acetyl-CoA to CO_2, generating NADH and $FADH_2$ used to generate energy in oxidative phosphorylation. The inputs for each molecule of acetyl-CoA include three molecules of NAD^+, one FAD, and one GDP + Pi.

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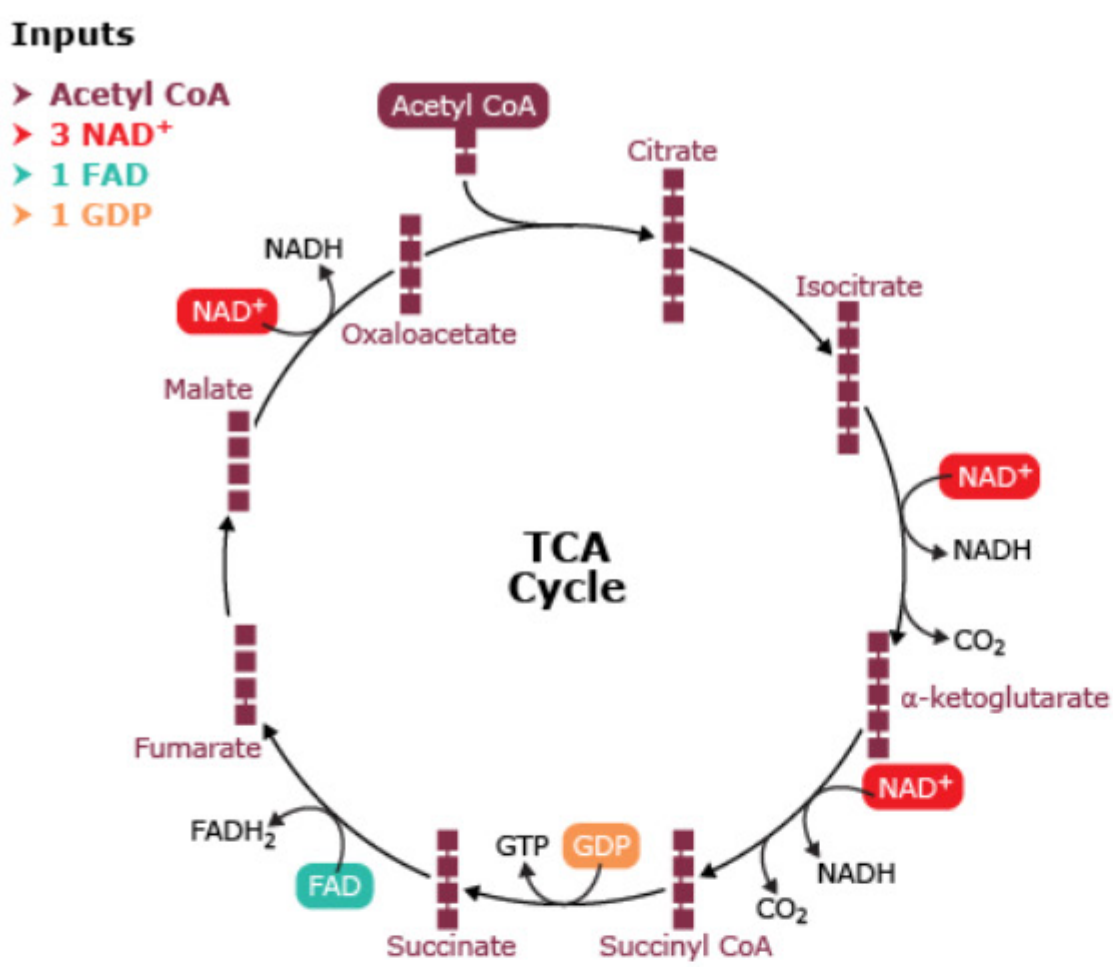
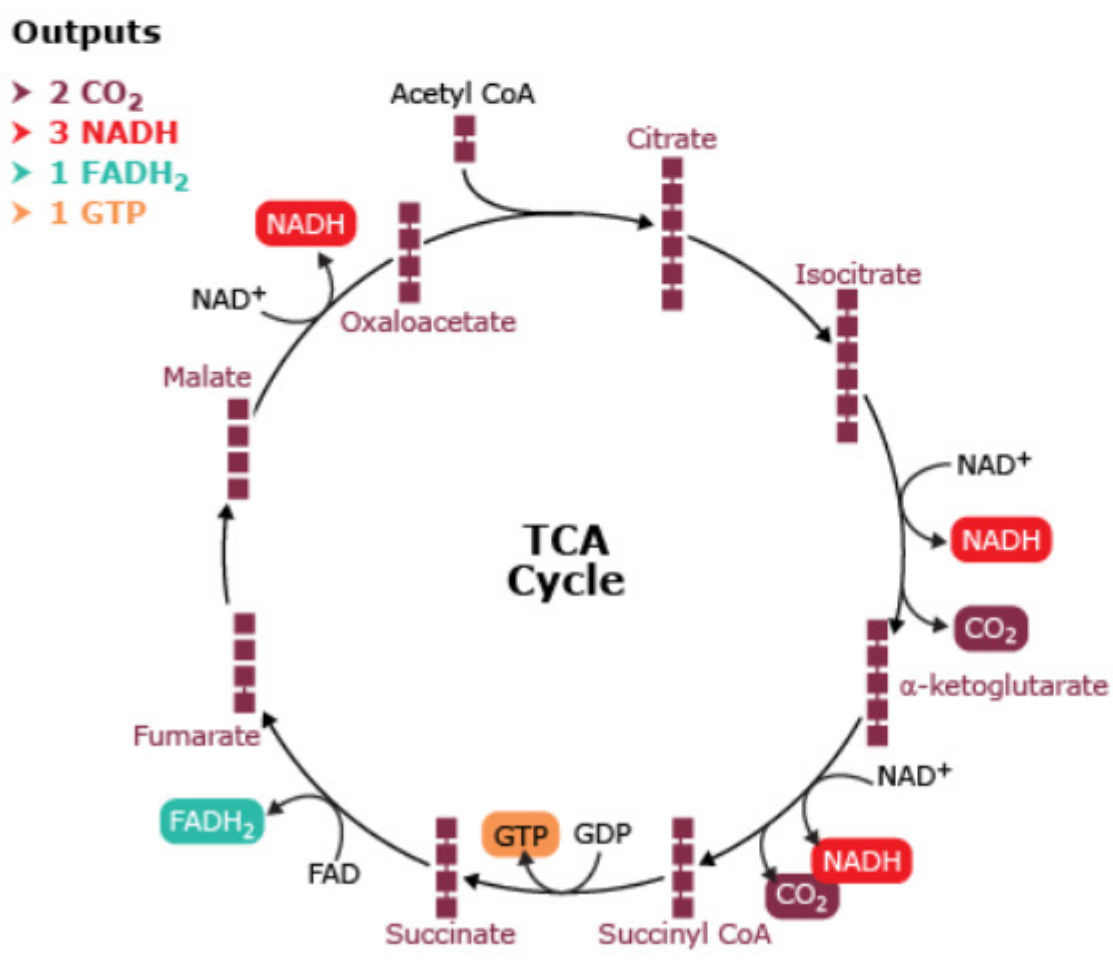


▲ **Figure 10–1.3A** Inputs to the TCA Cycle

The outputs for each molecule of acetyl-CoA are two CO_2, three NADH, one $FADH_2$, and one GTP.



▲ **Figure 10–1.3B** Outputs of the TCA Cycle

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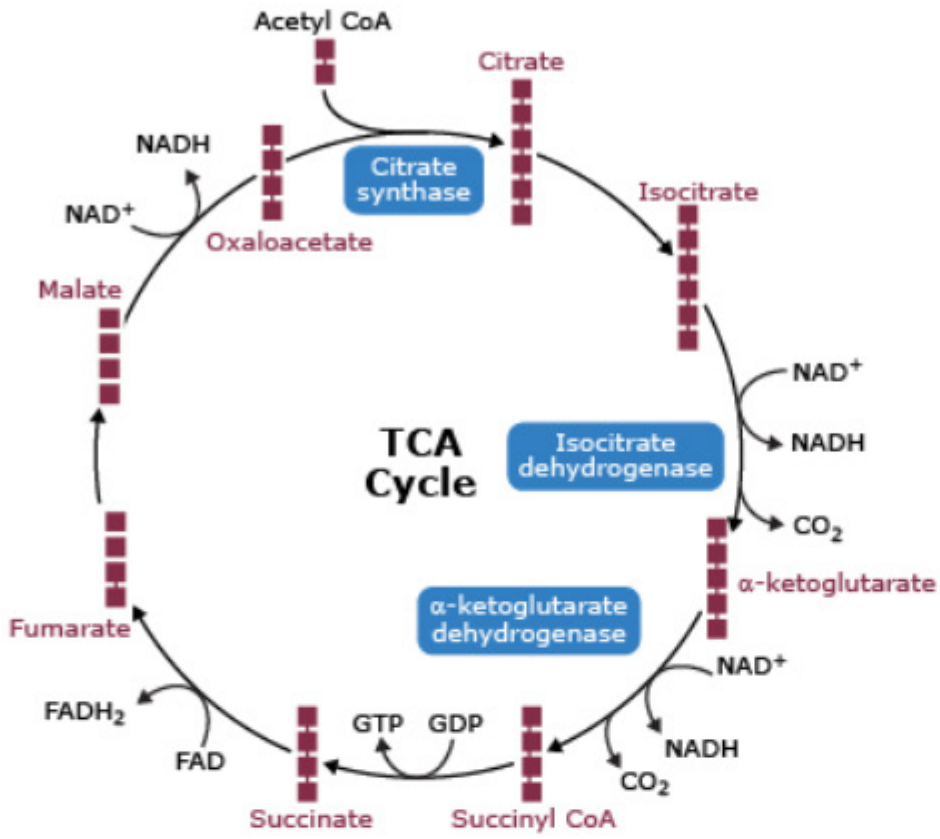
1.3.2 TCA Cycle Regulation

The TCA cycle is regulated to maintain energy homeostasis. In general, it is activated when energy needs are high, as indicated by high ADP/ATP or NAD^+/NADH ratios. It is inhibited when these ratios are low, indicating lower energy needs. Regulation of the TCA cycle occurs at three key steps:



▲ **Figure 10–1.3C** Regulation of the TCA Cycle

Citrate synthase catalyzes the first step of the TCA cycle, the condensation of acetyl-CoA and oxaloacetate to form citrate. This combines a 2-carbon and 4-carbon molecule to form a 6-carbon molecule. ATP is an allosteric inhibitor of citrate synthase. It increases the K_M of the enzyme for acetyl-CoA.

Isocitrate dehydrogenase converts the 6-carbon isocitrate to the 5-carbon α-ketoglutarate, producing the first CO_2 molecule of the cycle. It also generates a molecule of NADH. Similar to citrate synthase, ATP is an allosteric inhibitor of this enzyme, which ADP activates. NADH is a competitive inhibitor, competing for binding of NAD^+.

α-Ketoglutarate dehydrogenase converts the 5-carbon α-ketoglutarate to the 4-carbon succinyl-CoA, producing the second CO_2 molecule. It also generates a molecule of NADH. This enzyme is feedback inhibited by two of its products, NADH and succinyl CoA.

Important Concept

The TCA cycle is regulated at three steps:

1. Citrate synthase
2. Isocitrate dehydrogenase
3. α-ketoglutarate dehydrogenase

1.4 TCA Cycle in Metabolic Integration

Intermediates of the TCA cycle can be substrates for other biosynthetic pathways. We will see in chapter 9 how oxaloacetate and its precursor are involved in gluconeogenesis. In addition, oxaloacetate and α-ketoglutarate are precursors for the synthesis of certain amino acids. Furthermore, succinyl-CoA and fumarate are products of the degradation of certain amino acids. Thus, the TCA cycle serves as an intermediary between glucose and amino acid metabolic pathways. Through this cycle, amino acids can be broken down to generate gluconeogenic precursors, and glucose metabolism can support amino acid synthesis.

Important Concept

The TCA cycle serves as an intermediary between glucose, urea cycle amino acid metabolic pathways.

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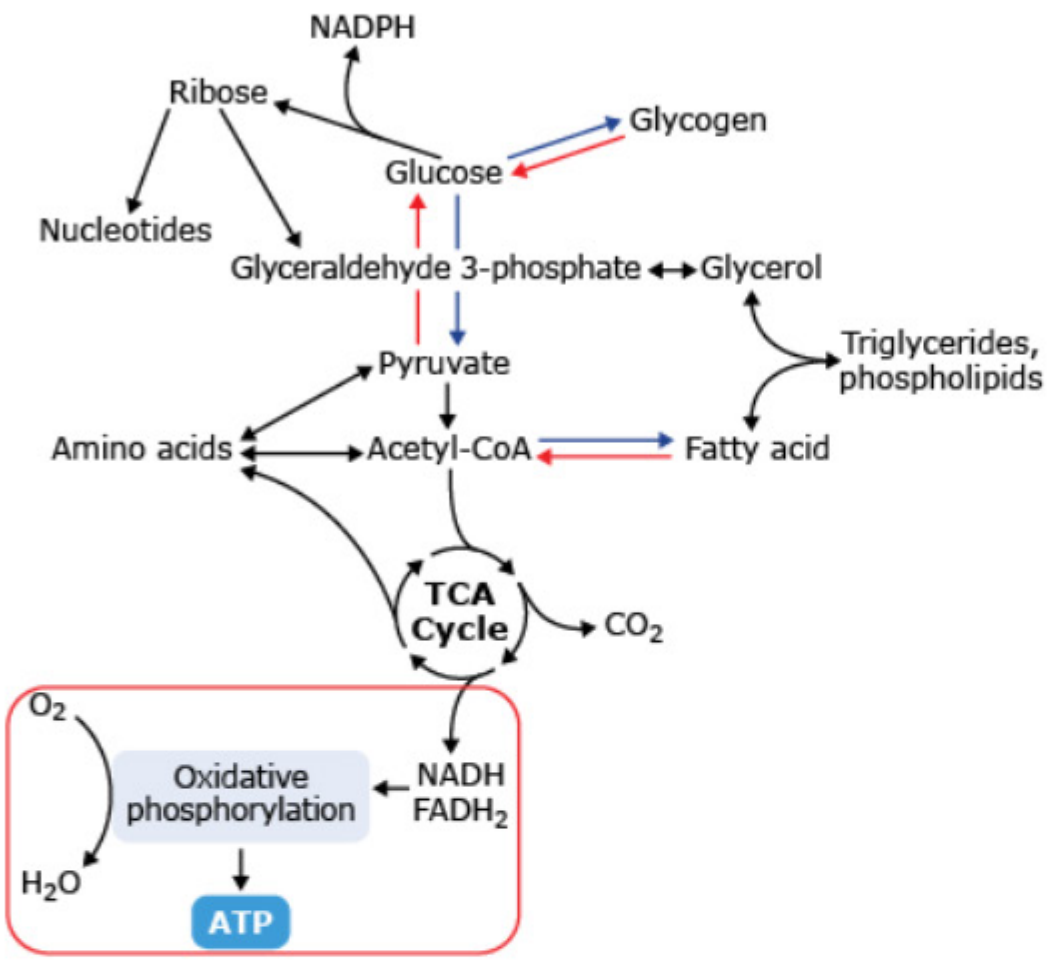
2 Oxidative Phosphorylation

Oxidative phosphorylation is the endpoint in metabolism of glucose and other fuels to generate energy. It consumes the reducing equivalents generated in glycolysis and the TCA cycle (NADH and $FADH_2$) and uses that energy in the presence of oxygen to produce ATP.



▲ **Figure 10–2.0** Role of Oxidative Phosphorylation in Glucose Metabolism

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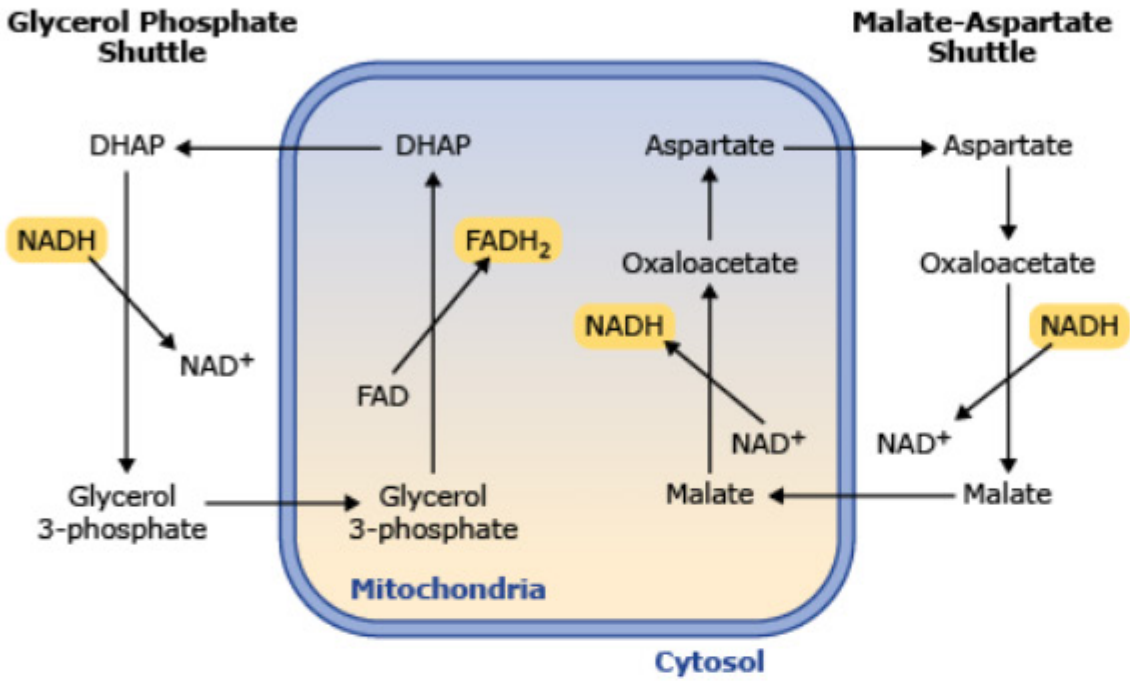
2.1 NADH Transport

Oxidative phosphorylation occurs in the inner mitochondrial membrane and utilizes NADH and $FADH_2$ in the mitochondrial matrix. However, glycolysis and ethanol metabolism generate NADH in the cytosol. There is not a mitochondrial transporter for NADH. Thus, more complex mechanisms are required to transport NADH across the inner mitochondrial membrane.



▲ **Figure 10–2.1** Glycerol Phosphate and Malate-Aspartate Shuttles

2.1.1 Glycerol Phosphate Shuttle

This is the primary mechanism for mitochondrial NADH transport in most tissues. Dihydroxyacetone phosphate (DHAP) is converted to glycerol 3-phosphate, oxidizing NADH to NAD^+. Glycerol 3-phosphate is transported into the mitochondria, where it is converted back to DHAP, generating a molecule of reduced $FADH_2$. DHAP is then transported back into the cytosol. The net result is exchange of cytosolic NADH to mitochondrial $FADH_2$. All other components are recycled. There is some energy loss in this process. This is because $FADH_2$ has less reducing power than NADH, as will be shown below.

2.1.2 Malate-Aspartate Shuttle

In the heart and liver, there is a second mechanism for mitochondrial NADH transport. In the cytosol, oxaloacetate is converted to malate, oxidizing NADH to NAD^+. Malate is transported into the mitochondrial matrix, where it is converted back to oxaloacetate (this is the final reaction of the TCA cycle), producing a molecule of reduced NADH from NAD^+ in the process. Oxaloacetate is converted to the amino acid aspartate and transported back to the cytosol where it is converted back to oxaloacetate. The net result is exchange of cytosolic NADH to mitochondrial NADH, which is more efficient than the glycerol phosphate shuttle because there is no loss of reducing power. All other components are recycled.

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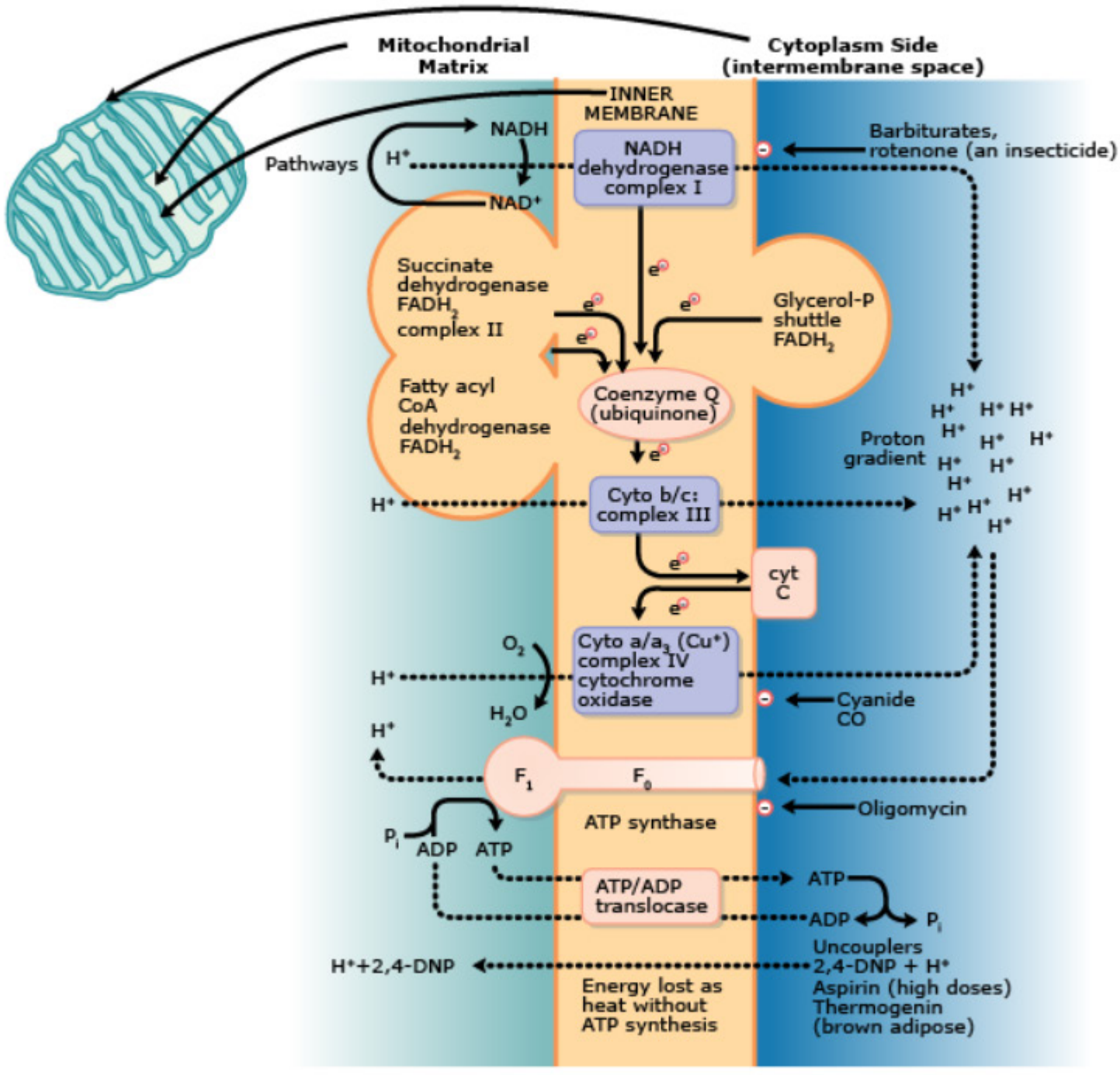
2.2 Electron Transport Chain (ETC)

The energy in NADH and $FADH_2$ is stored as *reduction potential*, the ability to reduce or donate electrons to other compounds. The electron transport chain consists of a series of protein complexes associated with the inner mitochondrial membrane. These proteins catalyze a series of oxidation-reduction reactions in which electrons from NADH and $FADH_2$ are passed from compounds of higher reduction potential to compounds of lower reduction potential. The primary goal of the ETC is the generation of a proton gradient between the matrix and the intermembrane space of the mitochondria. ATP synthase uses this gradient to phosphorylate ADP to ATP.



▲ **Figure 10–2.2** Electron Transport Chain

NADH donates electrons to protein complex I, through coenzyme Q to complex III, and via cytochrome c to complex IV. $FADH_2$ donates electron to complex II, followed by complexes III and IV. The final electron acceptor is molecular oxygen (O_2), which is converted to water (H_2O). These are energetically favorable reactions, such that free energy is liberated at each step. This energy is used to pump protons (H^+) out of the mitochondrial matrix into the intermembrane space.

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2.3 ATP Synthase

The net result of the electron transport chain is the establishment of an electrical and chemical gradient across the inner mitochondrial membrane. The intermembrane space has a higher proton concentration (lower pH) and is more positive than the matrix. Thus, if allowed, the protons tend to flow down this gradient from the intermembrane space to the matrix. This is electrochemical potential energy.

ATP synthase is a protein complex on the inner mitochondrial membrane that consists of two essential parts. The F_0 portion is in the membrane and the F_1 portion sits in the matrix. ATP synthase allows the protons to flow down the electrochemical gradient from the intermembrane space into the matrix. This is an energetically favorable reaction and the liberated free energy is harnessed to convert ADP to ATP.

The stoichiometry of the reaction is as follows. One molecule of NADH has reducing power sufficient to generate approximately three molecules of ATP. $FADH_2$ has less reducing power, and generates only two ATP per molecule.

Important Concept

For ATP synthase:

1 NADH makes ~ 3 ATP

1 $FADH_2$ makes ~ 2 ATP

2.4 ATP Transport

ATP generated in the mitochondria needs to be utilized throughout the cell. ATP is exported from the mitochondria by an ADP/ATP antiporter that moves one ATP out of the mitochondrion in exchange for one ADP entering the mitochondrion. Both are moving down their respective concentration gradients such that no exogenous energy input is required.

2.5 Uncoupling

Another function of the proton gradient created by the electron transport chain is heat generation. If protons are allowed to flow down their concentration gradient outside of ATP synthase, the electrochemical energy is released as heat. This is known as *uncoupling*.

In mammals, this process occurs in a type of adipose tissue known as brown fat. It gets its color from an abundance of mitochondria. It is particularly abundant in hibernating or cold-adapted animals, but is also seen in human infants. The mitochondria express a protein called *thermogenin*, or uncoupling protein 1 (UCP1), which is a proton channel in the inner mitochondrial membrane. Cold-activated signals from the hypothalamus cause release of norepinephrine, which stimulates fatty acid catabolism as a source of energy for the electron transport chain, and activates thermogenin. Protons flow through thermogenin, generating heat. Uncoupling proteins also occur in other tissues, such as pancreatic β cells, where they are thought to play a role in regulating insulin release in response to increased glucose concentration.

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2.6 Inhibitors of Oxidative Phosphorylation

There are a number of drugs and toxins that inhibit oxidative phosphorylation at different sites:

- **Complex I:** Rotenone, barbiturates, mercurials
- **Complex III:** Antimycin
- **Complex IV:** Cyanide, azides, carbon monoxide
- **ATP synthase:** Oligomycin

Dinitrophenol is a synthetic compound that allows free flow of protons across the inner mitochondrial membrane. As such, it acts as an uncoupler, reducing ATP synthesis and generating heat. In high concentrations, it can cause fatal hyperthermia.

2.7 Calculating ATP generation

Calculating total ATP production from total metabolism of a single molecule of glucose provides a useful summary of the processes involved in metabolism of glucose from glycolysis through the production of ATP.

Glycolysis consumes two and generates four molecules of ATP. It also produces two reduced NADH. The ATP generated in oxidative phosphorylation by the NADH depends on the pathway they take into the mitochondria. In most tissues, they will follow the glycerol phosphate shuttle and be converted to two $FADH_2$, which produce two ATP each (total of four). In the heart and liver, the malate-aspartate shuttle exchanges without conversion of the NADH, which produces three ATP each (total of six).

Pyruvate dehydrogenase generates a molecule of NADH for each pyruvate (two total), each of which produces three ATP (six total).

The TCA cycle turns twice, once for each acetyl-CoA, and generates two GTP (equivalent of two ATP), six NADH (generating 18 ATP), and two $FADH_2$ (generating four ATP).

In summary, one molecule of glucose generates:

Glycolysis			2 ATP
	2 NADH	=	4–6 ATP
Pyruvate dehydrogenase	2 NADH	=	6 ATP
TCA Cycle	2 GTP	=	2 ATP
	6 NADH	=	18 ATP
	2 $FADH_2$	=	4 ATP
Total			36–38 ATP

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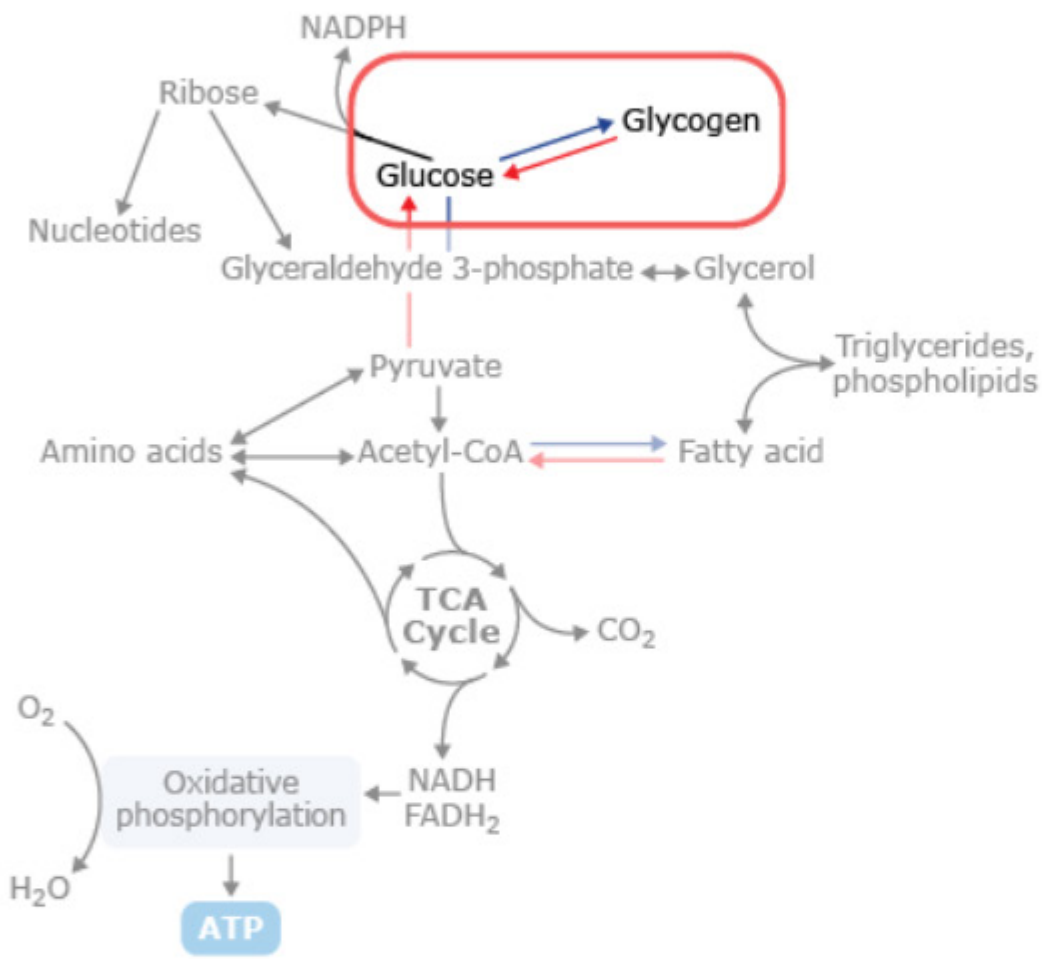
Glycogen Synthesis and Glycogenolysis

1 Glycogen

Glycogen is the major storage form of glucose in humans. Glycogen synthesis converts excess glucose to glycogen for storage in the fed state. Glycogen degradation releases glucose from stored glycogen to provide additional glucose in the fasting state.



▲ **Figure 11–1.0** Role of Glycogen in Glucose Metabolism

Glycogen is stored primarily in two sites. The liver stores glycogen to maintain blood glucose during a short fast. Muscle stores glycogen to provide an additional source of fuel during exercise.

USMLE® Key Concepts

For Step 1, you must be able to:

- Describe the role of glycogen synthesis and glycogenolysis in metabolism.
- Identify the structure of glycogen and describe its effects on metabolism.
- Explain the mechanisms of glucagon and insulin in blood glucose regulation.
- Identify common glycogen storage diseases.
- Describe the hexose monophosphate pathway and the purpose of its key products in cells.

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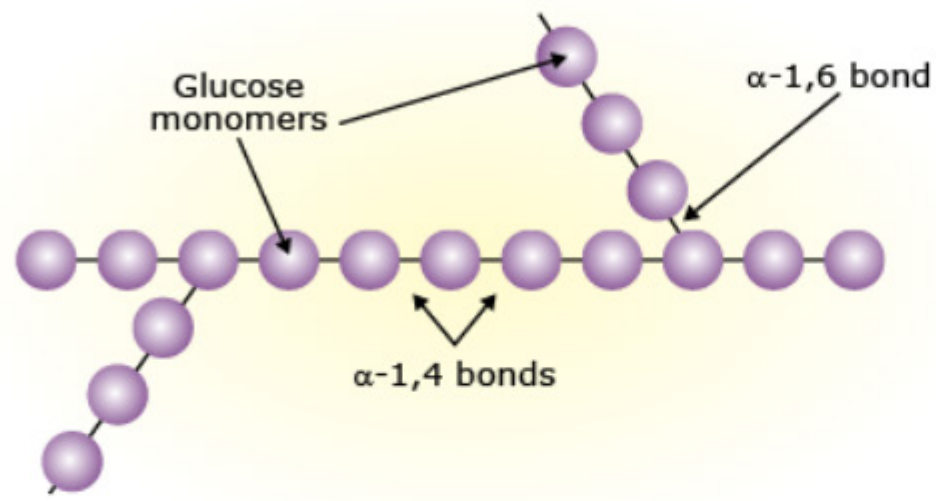
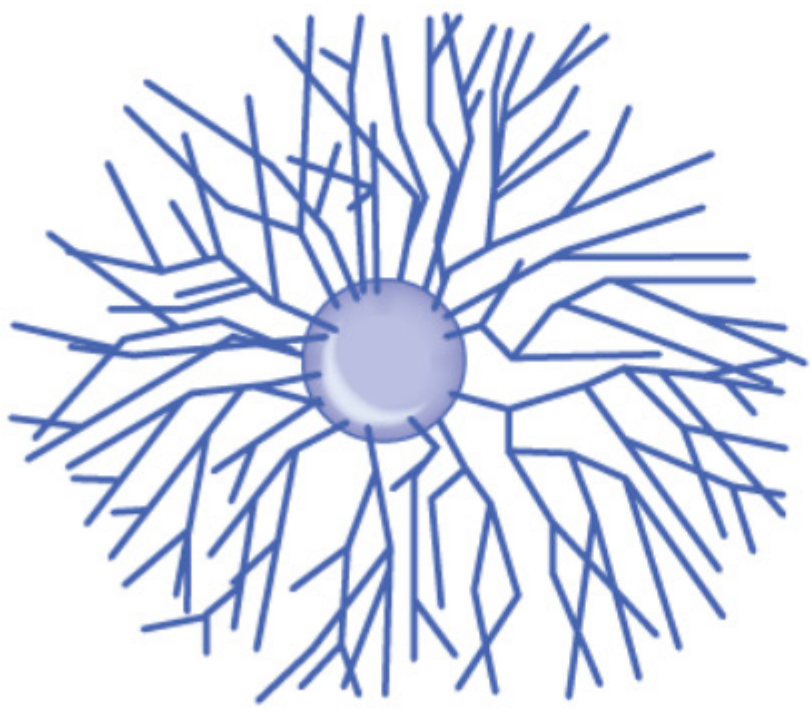
1.1 Glycogen Structure

As stated previously, glycogen is a polymer of glucose. In most cases, the individual glucoses are connected by *α-1,4 glycosidic bonds*. These are covalent bonds between the oxygen groups at carbon 1 of one glucose molecule and carbon 4 of another glucose molecule. The problem with this arrangement is that synthesis and degradation are relatively slow and inefficient if they can only work at one end at once. To remedy this, α-1,6 glycosidic bonds are also created periodically as points from which additional polymers can branch off. This creates a multitude of ends from which glucose molecules can be rapidly hydrolyzed when needed.



▲ **Figure 11–1.1A** Glycogen Structure

In its storage form, glycogen is organized into granules around a protein core, with the traditional glycogen chains (above) radiating and branching outward.



▲ **Figure 11–1.1B** A Glycogen Granule

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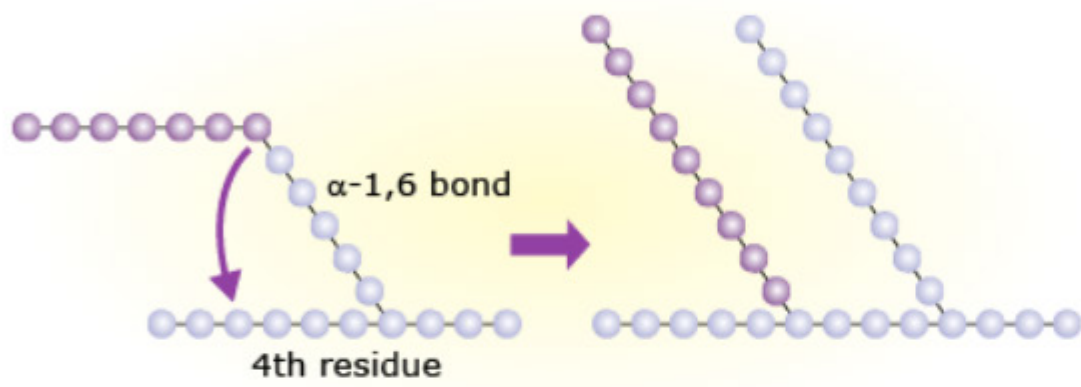
1.2 Glycogen Synthesis

The synthesis of glycogen is catalyzed by an enzyme called *glycogen synthase*. The primary substrate for the reaction is UDP-glucose, a molecule of glucose covalently bonded to the nucleotide UDP. Glycogen synthase takes the glucose monomers from UDP glucose and adds them to the end of the growing glycogen chain by creating new α-1,4 glycosidic bonds.

The branches of the glycogen molecule are created by the branching enzyme. This enzyme removes polysaccharides of seven glucoses in length and creates branch points by synthesizing α-1,6 glycosidic bonds.



▲ **Figure 11–1.2** Action of Branching Enzyme in Glycogen Synthesis

Looking Back

UDP glucose is also an intermediate in the metabolism of galactose and the formation of glycoproteins.

Looking Ahead

UDP glucose is also a precursor used for the synthesis of lipopolysaccharides and glycosphingolipids.

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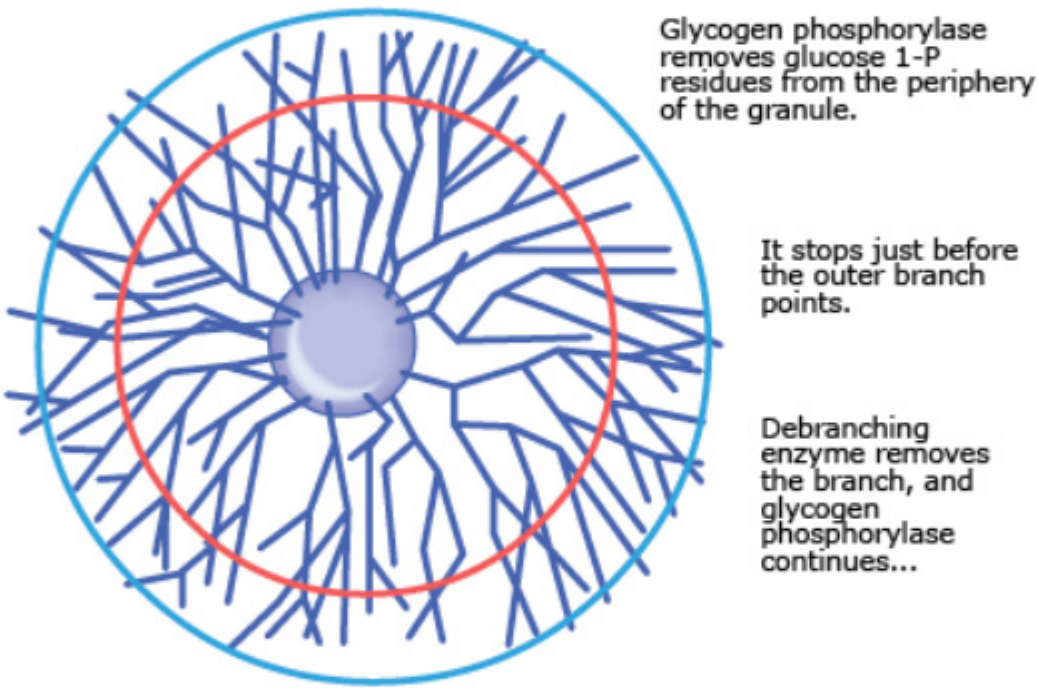
2 Glycogenolysis

2.1 Overview of Glycogenolysis

Breakdown or degradation of glycogen is called *glycogenolysis*. This process is essentially a reversal of glycogen synthesis that is catalyzed by *glycogen phosphorylase*. This is the rate-limiting enzyme, which breaks the α-1,4 glycosidic bonds, releasing the individual glucose units as glucose 1-phosphate (G1P). G1P is converted to glucose 6-phosphate for glycolysis.

In muscle, glucose 1-phosphate enters glycolysis to be metabolized for energy production. In the liver, it is converted to glucose by glucose 6-phosphatase and released into circulation. The α-1,6 glycosidic bonds at the branch points are broken by the *debranching enzyme*.



▲ **Figure 11–2.1** Enzymatic Breakdown of a Glycogen Granule

2.2 Regulation of Glycogen Metabolism

The purpose of glycogen synthesis is to store excess glucose in the fed state. Glycogenolysis, or glycogen degradation, releases glucose from the liver to maintain blood levels in the fasting state or it metabolizes glycogen for energy in exercising muscle.

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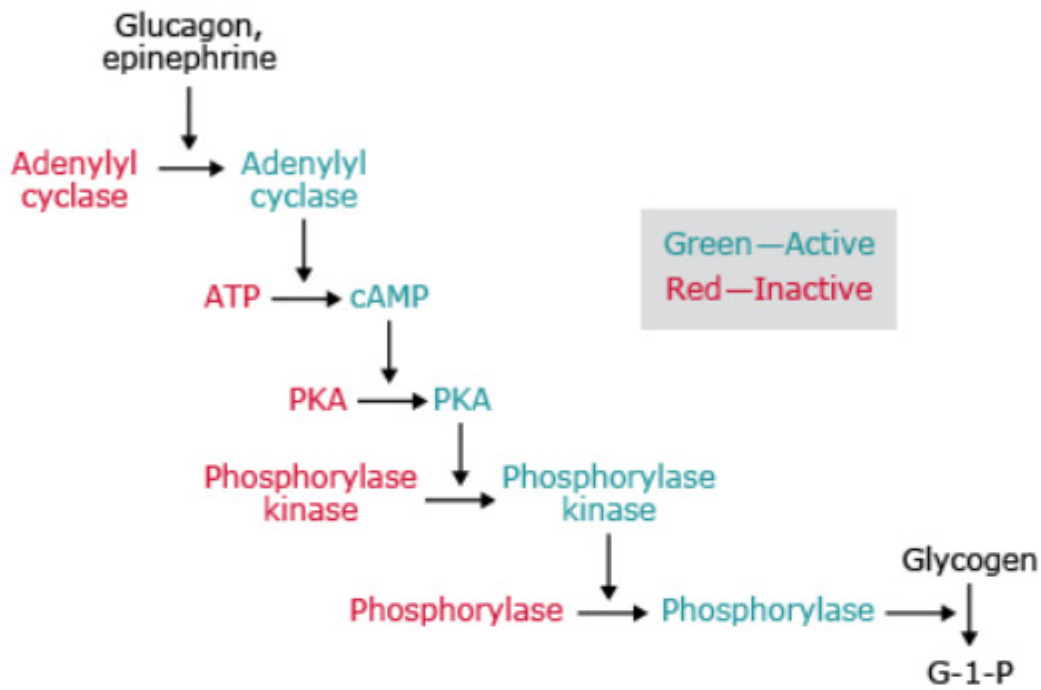
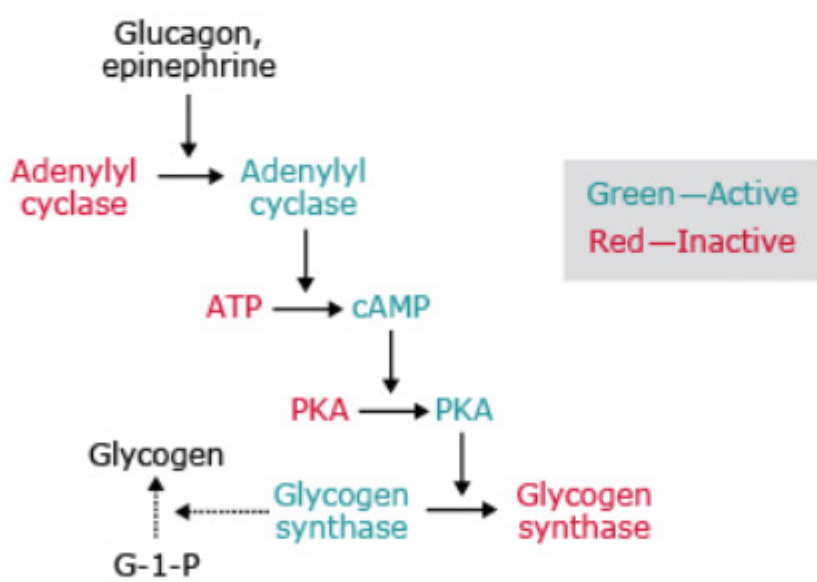
2.2.1 Glucagon and Catecholamines

Glucagon and catecholamines stimulate glycogenolysis while inhibiting glycogen synthesis in the fasting state. These hormones regulate the glucagon pathways through a signaling cascade. Their G-protein-associated receptors cause activation of adenylyl cyclase, increasing cAMP concentration. cAMP activates protein kinase A (PKA), which activates phosphorylase kinase. This, in turn, activates glycogen phosphorylase, stimulating glycogen degradation.



▲ **Figure 11–2.2A** Glucagon Signaling Cascade

At the same time, PKA phosphorylates glycogen synthase, causing its inactivation. This ensures that glycogen degradation and glycogen synthesis are mutually exclusive; that is, only one of the pathways is activated at a time.



▲ **Figure 11–2.2B** Deactivation of Glycogen Synthase by Glucagon

Phosphorylation cascades are important in the regulation of this pathway because they produce signal amplification. The binding of a single glucagon to a cell receptor can activate multiple PKA enzymes. Each PKA can stimulate multiple adenylyl cyclases, and so forth. At each step the signal is amplified, stimulating a substantial and rapid response to glucagon.

Important Concept

Glucagon causes *phosphorylation:*

- Glucagon stimulates glycogenolysis by phosphorylating glycogen phosphorylase.
- Glucagon blocks glycogen synthesis by phosphorylating glycogen synthase.

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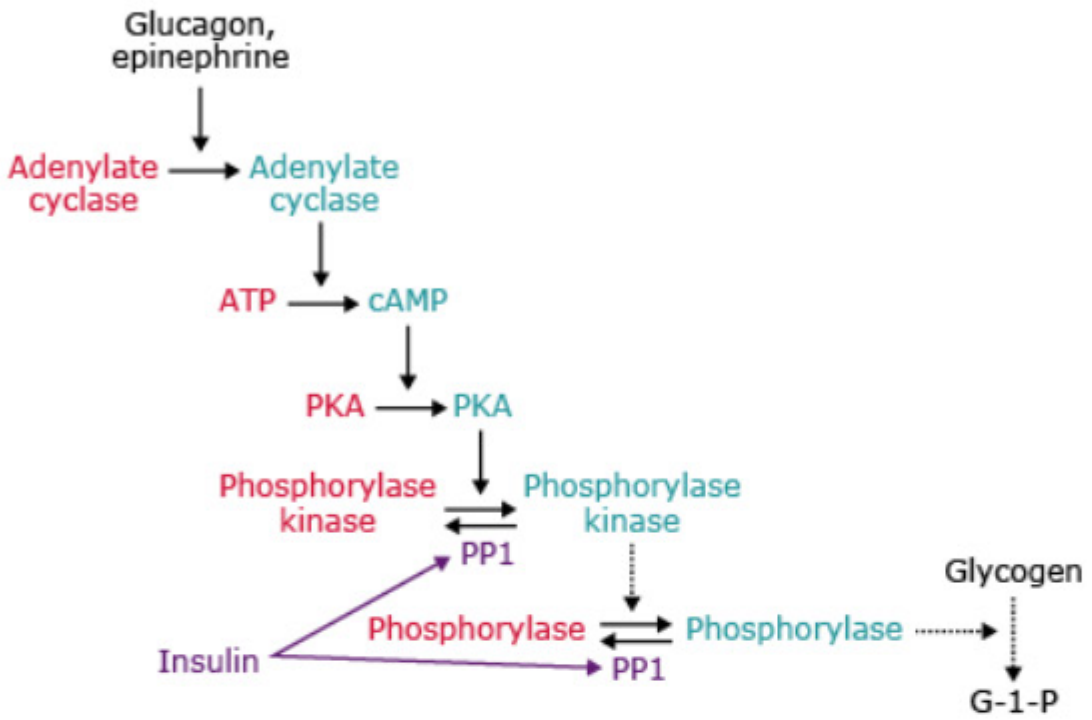
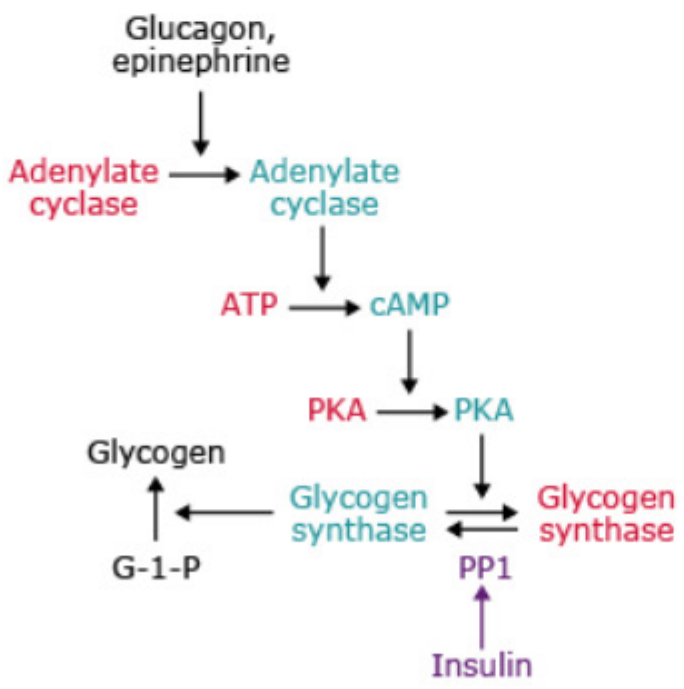
2.2.2 Insulin

Insulin stimulates glycogen synthesis and inhibits glycogenolysis in the fed state—precisely the opposite of the effects of glucagon. Insulin stimulates protein phosphatase 1 (PP1) through a kinase cascade that begins with the insulin receptor. These dephosphorylate and inactivate both phosphorylase kinase and glycogen phosphorylase, blocking glycogen degradation.



▲ **Figure 11–2.2C** Insulin Signaling Cascade

At the same time, PP1 dephosphorylates glycogen synthase, activating glycogen synthesis. Similar to the effects of glucagon, this ensures the mutual exclusivity of glycogen synthesis and degradation.



▲ **Figure 11–2.2D** Activation of Glycogen Synthase by Insulin

Important Concept

Insulin causes *dephosphorylation:*

- Insulin stimulates glycogen synthesis by dephosphorylating glycogen synthase.
- Insulin blocks glycogenolysis by dephosphorylating phosphorylase kinase and glycogen phosphorylase.

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2.3 Glycogen Storage Diseases

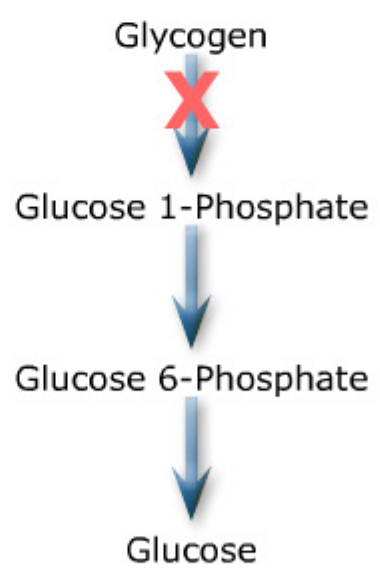
There are at least 10 subtypes of glycogen storage disease that are differentiated by the type of enzyme defect and the tissue specificity. These can be divided into three general categories.

- **Hepatic Forms:** Types I, III, VI, and VIII
- **Muscle Forms:** Types V and VII
- **Miscellaneous Forms:** Types II and IV

▼**Table 11–2.3** Common Glycogen Storage Diseases

Type	Deficient Enzyme	Tissue	Clinical Features	Glycogen Structure
I: von Gierke	Glucose 6-phosphatase	Liver	Severe hypoglycemia, hepatomegaly, lactic acidosis, hyperlipidemia, hyperuricemia, short stature, doll-like faces, protruding abdomen, emaciated extremities	Normal
II: Pompe	Lysosomal α-1,4 glucosidase	Misc.	• Cardiomyopathy, muscle weakness • Without treatment leads to early death • Treatment: Enzyme replacement therapy	Normal
III: Cori	Debrancher enzyme	Liver	Mild hypoglycemia, liver enlargement	• Short outer branches • Single glucose residue at branch
IV: Andersen (amylopectinosis)	Brancher enzyme	Misc.	Infantile hypotonia, cirrhosis, death by 1 year of age	Very few branches
V: McArdle	Myophosphorylase	Muscles	Muscle weakness and cramping on exercise (initial phase)	Normal
VI: Hers	Liver glycogen phosphorylase	Liver	Mild fasting hypoglycemia, hepatomegaly	Normal

McArdle syndrome (type V) is the classic example of the muscle forms of glycogen storage disease. It is caused by a mutation causing the deficiency of a muscle-specific isoform of glycogen phosphorylase. Thus, during exercise, affected individuals are unable to efficiently mobilize glucose through glycogenolysis and cannot produce sufficient energy to maintain muscle activity. This leads to painful muscle cramps. Some of the myocytes die from insufficient energy and release myoglobin, which is cleared by the kidneys, leading to myoglobinuria, or dark urine after exercise. McArdle syndrome has a late onset, usually after age 20, and patients are otherwise healthy and have normal longevity.



▲**Figure 11–2.3** Disruption of Glycogen Metabolism

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The most common form of hepatic glycogen storage disease is von Gierke disease (type I). Type IA von Gierke disease is caused by a deficiency of glucose 6-phosphatase. Type IB von Gierke disease is less common and is caused by a deficiency of the glucose 6-phosphate microsomal transporter, which transports glucose into the endoplasmic reticulum, where glucose 6-phosphatase is located. In either case, glucose 6-phosphate produced by glycogenolysis cannot be converted to glucose for transport out of the liver. The excess glucose 6-phosphate feeds back to inhibit further glycogenolysis, causing glycogen to build up in the liver.

The clinical features of von Gierke disease typically occur in infants and include hepatomegaly and renomegaly (enlarged kidneys) from glycogen accumulation. Patients frequently exhibit failure to thrive and have hypoglycemia due to the inability to mobilize glycogen (or activate gluconeogenesis), which can lead to ketosis and/or seizures.

Von Gierke disease is treated with frequent feeding and a high-carbohydrate diet. This decreases the patient's dependence on glycogen metabolism. If treated properly, patients can avoid most of the severe consequences of the disease.

The most common of the miscellaneous glycogen storage diseases is Pompe disease (type II). This is caused by a defect in an enzyme called α-1,4 glucosidase or acid maltase, which breaks down glycogen in lysosomes. The result is a massive accumulation of glycogen in many tissues, including the liver, heart, and skeletal muscle. The heart accumulation is particularly harmful in this disease, generally leading to death from cardiorespiratory failure at an early age.

Also in the miscellaneous category, but less common, is Andersen disease (type IV). It is unique because it is the only glycogen storage disease caused by a defect in glycogen synthesis. It results from a defect in the branching enzyme, leading to the accumulation of long, unbranched chains of glycogen. As indicated earlier, this makes glycogenolysis very inefficient. Glycogen accumulates in the hepatocytes, causing progressive cirrhosis and early death from liver failure.

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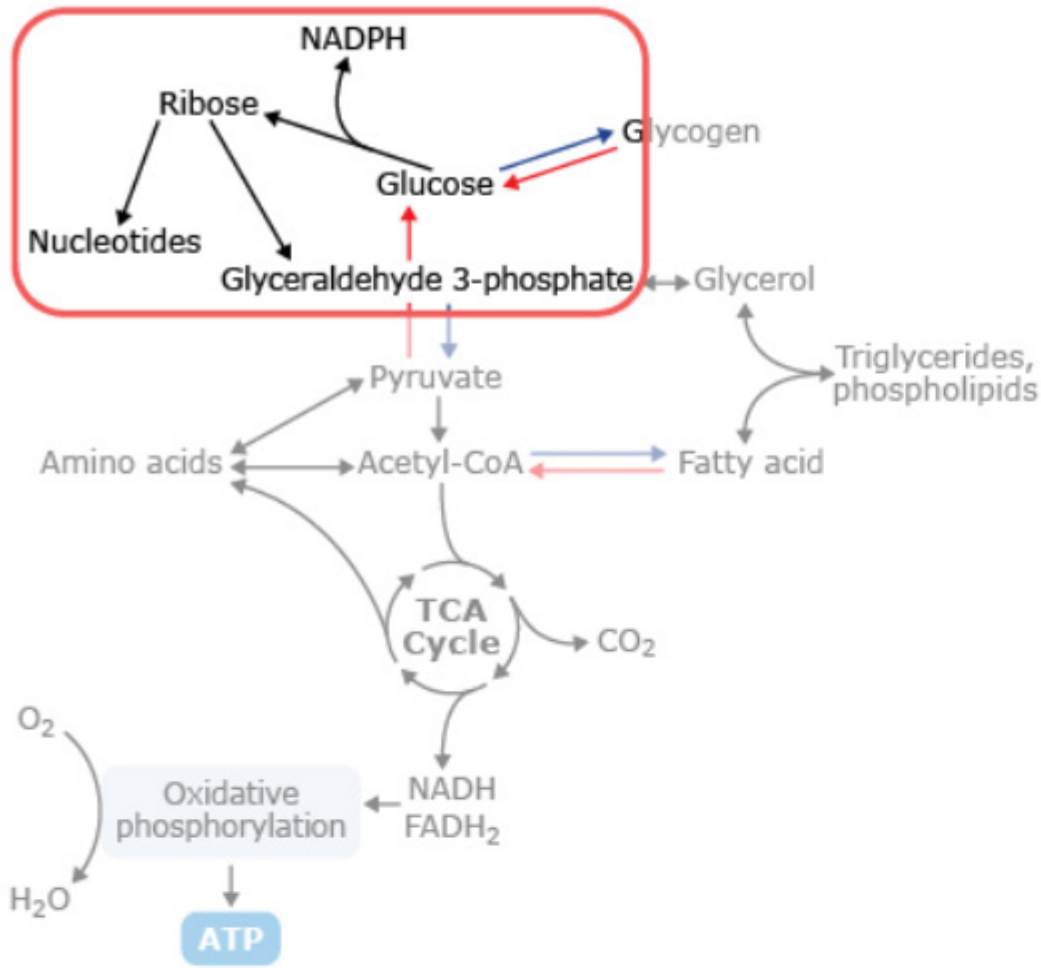
3 The Hexose Monophosphate Shunt



▲ **Figure 11–3.0** Role of Hexose Monophosphate Shunt in Glucose Metabolism

3.1 NADPH

NADPH is a reducing agent similar to NADH, but it has a fundamentally different role in metabolism. Rather than using its reduction potential to drive energy production, NADPH uses its reduction potential to drive redox reactions in biosynthetic pathways, including:

- Glutathione production
- Fatty acid synthesis and desaturation
- Cholesterol and sphingolipid synthesis
- Reduction of dihydrofolate

NADPH is produced in cells by two different pathways:

- Hexose monophosphate shunt
- Citrate shuttle (discussed in chapter 12, "Lipid Metabolism and Catabolism")

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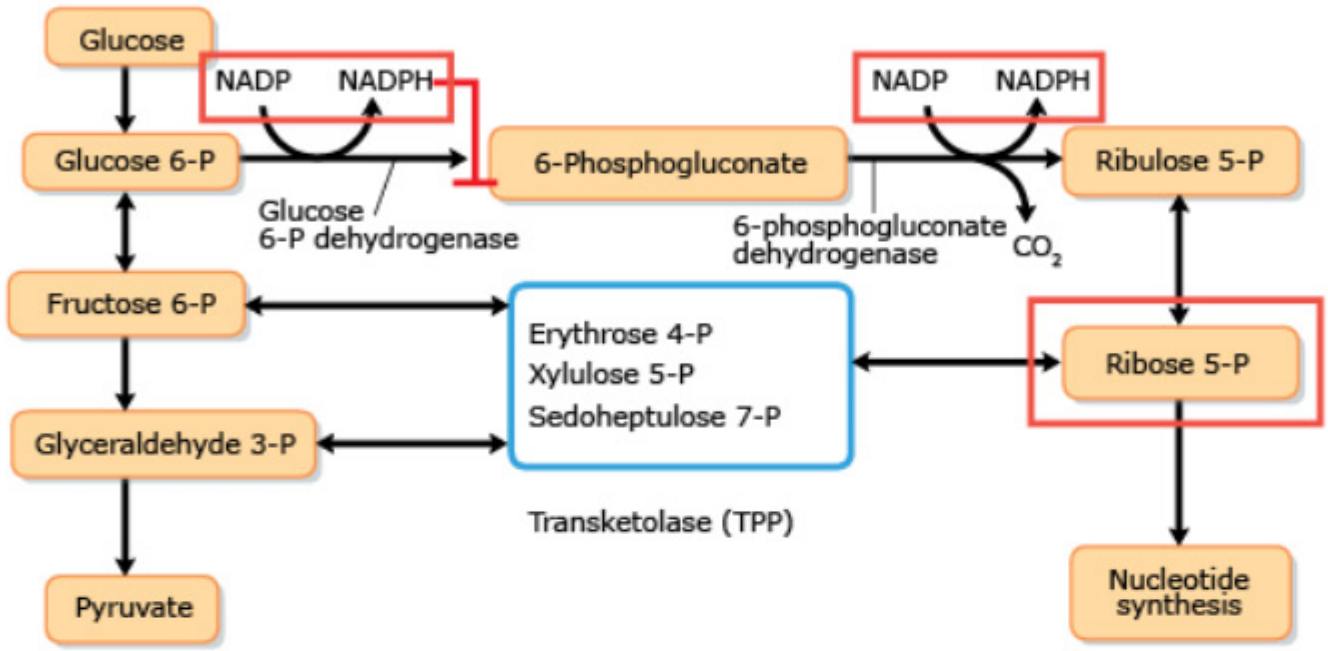
3.2 Hexose Monophosphate Shunt

The hexose monophosphate shunt (or pentose phosphate pathway) is a parallel pathway to glycolysis that takes glucose 6-phosphate (G-6-P), produced by glycolysis, and converts it through a different series of reactions to fructose 6-phosphate and glyceraldehyde 3-phosphate while generating NADPH in the process.

The first step is the conversion of G-6-P to ribose 5-phosphate (R-5-P), which generates two NADPH as shown in Figure 11–3.2A.



▲ **Figure 11–3.2A** Hexose Monophosphate Shunt

Glucose 6-phosphate dehydrogenase (G6PD) initiates this pathway, and comprises the rate-limiting and only regulated step in the whole pathway. G6PD is feedback inhibited by NADPH, which competes directly with NADP+ for the enzyme active site. Therefore, the NADPH/NADP+ ratio ensures that the cell only produces the needed amount of NADPH.

The R-5-P produced by the hexose monophosphate shunt has two potential fates:

- Substrate for the synthesis of nucleotides—it produces the ribose sugar backbone.
- Conversion through multiple steps to glycolytic intermediates (the shunt):

3 ribose 5-phosphate → 2 fructose 6-phosphate + glyceraldehyde 3-phosphate

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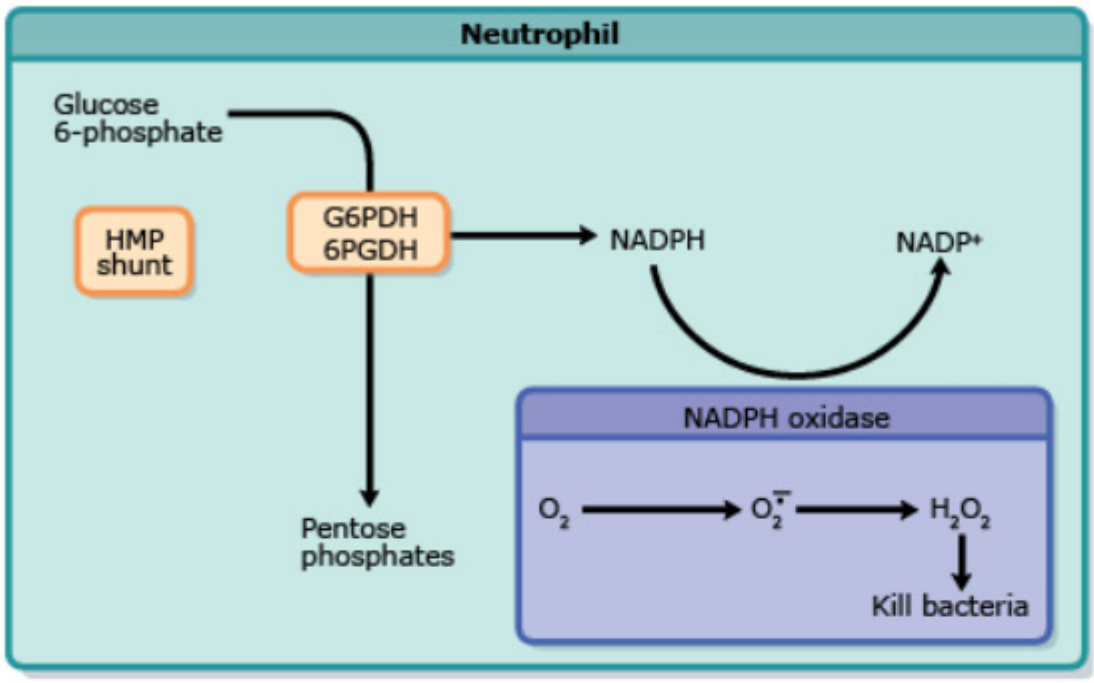
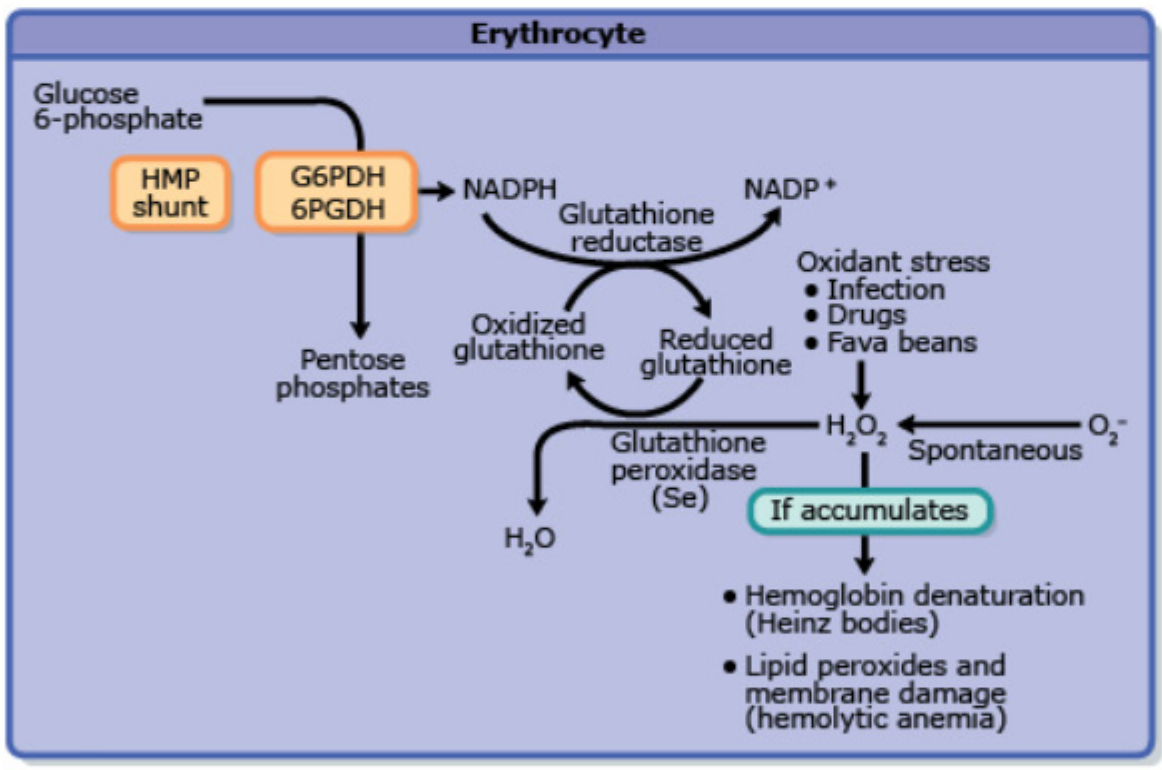
The hexose monophosphate shunt has different roles in certain cells. The most important examples are in neutrophils and erythrocytes, as shown in Figures 11–3.2B and C.



▲ **Figure 11–3.2B** Hexose Monophosphate Shunt in Neutrophils



▲ **Figure 11–3.2C** Hexose Monophosphate Shunt in Erythrocytes

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Clinical Application

Glucose 6-Phosphate Dehydrogenase Deficiency

Glucose 6-phosphate dehydrogenase (G6PD) deficiency is an X-linked recessive disorder most commonly seen in males of African or Mediterranean descent. It is caused by mutations in the G6PD gene that cause the protein to be unstable, significantly reducing its half-life. The disease most often manifests as episodic hemolytic anemia. This is due to reduced production of NADPH in red blood cells, the primary role of which is the regeneration of glutathione. Consequently, under conditions of oxidative stress, such as infection or exposure to oxidizing drugs (sulphonamides, nitrofurans, or anti-malarial drugs such as quinine, primaquine, or chloroquine), reduced glutathione levels are inadequate to reverse oxidative damage. This results in membrane defects and precipitates hemoglobin, which forms Heinz bodies. The defective red blood cells are cleared by macrophages in the spleen and liver, resulting in anemia.

An interesting facet of this disease is its concentration in red blood cells. No other cells in the body appear to be affected. There are three reasons for this:

1. Red blood cells are particularly prone to oxidative damage because they carry molecular oxygen, the source of oxygen free radicals.
2. Because they don't have mitochondria, red blood cells rely solely on the pentose phosphate shunt for NADPH production. Other cells can produce NADPH through the citrate shuttle that occurs in mitochondria.
3. Red blood cells can't synthesize new protein. In other cells, the unstable G6PD can be replaced by new protein synthesis, which does not occur in the anucleate red blood cells.

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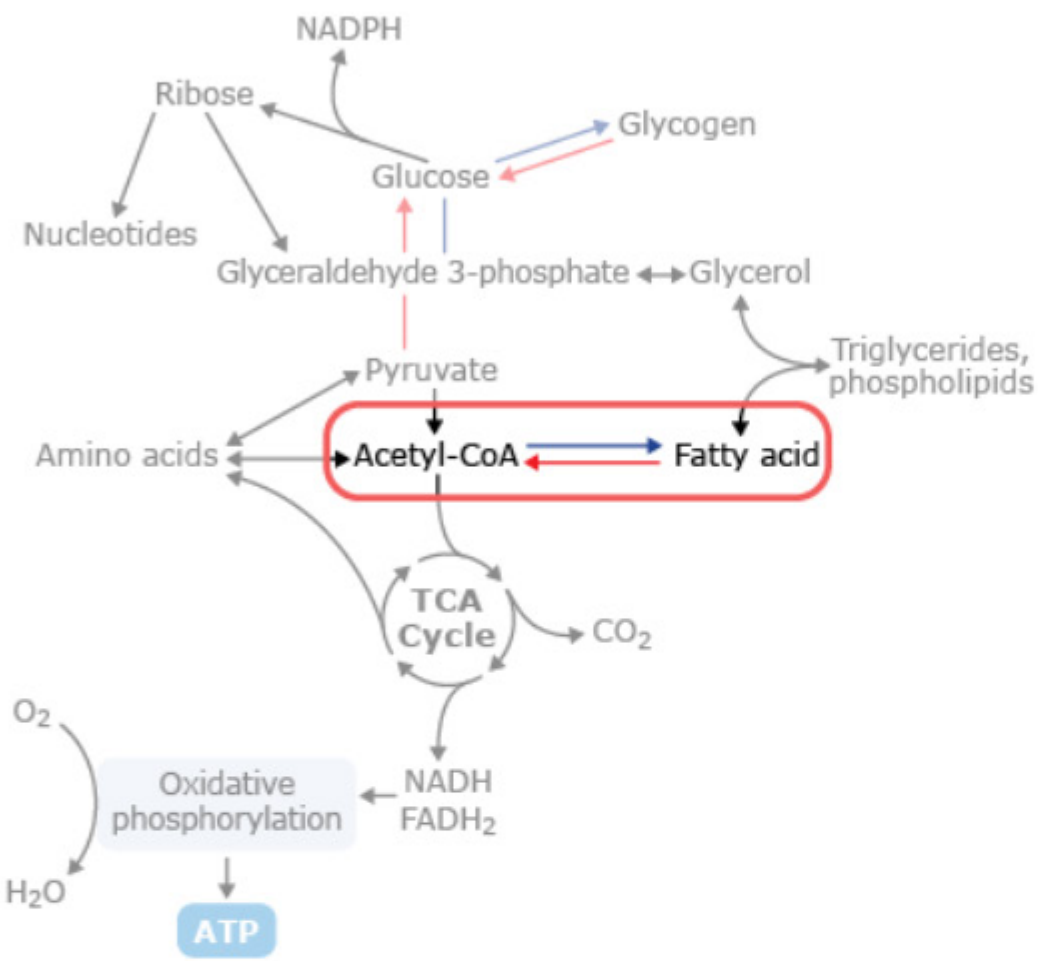
1 Overview of Lipid Synthesis



▲ **Figure 12–1.0** Lipid Synthesis in Glucose Metabolism

USMLE® Key Concepts

For Step 1, you must be able to:

- Explain the action and regulation of pathways of fatty acid synthesis.
- Describe the transport of triglycerides following dietary intake and their dissemination to the tissues.
- Identify and explain the biochemical basis of the primary hyperlipidemias and hypercholesterolemia.

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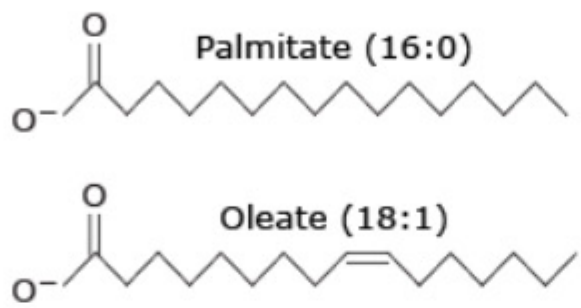
2 Fatty Acid Structure

Fatty acids are long-chain carboxylic acids consisting of a hydrocarbon acyl chain with a carboxyl group at one end. Some fatty acids are unsaturated; that is, they have double bonds in their acyl chains. Double bonds in natural fatty acids are in the *cis* configuration. *Trans* double bonds predominate in artificially produced foods and, along with saturated fatty acids, they are associated with an increased risk of atherosclerosis.



▲ **Figure 12–2.0** Saturated *(top)* and Unsaturated *(bottom)* Fatty Acids

Fatty acids are identified by using common names as well as numerical identifiers. For example, the nomenclature "oleate (18:1)" means that that the fatty acid in question has 18 carbons with 1 double bond, identifying oleate (oleic acid) as a monounsaturated fatty acid.

▼ **Table 12–2.0** Common Fatty Acids

Common Name	Numerical Identifiers
Lauric	12:0
Myristic	14:0
Palmitic	16:0
Stearic	18:0
Oleic	18:1
Linoleic	18:2
Linolenic	18:3
Arachidonic	20:4

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3 Fatty Acid Biosynthesis

Dietary glucose can be converted to fatty acids in the liver and sent to adipose tissue for storage. Fatty acids are synthesized *in the cytosol* from acetyl-CoA, which is generated in the mitochondria. Insulin promotes several steps in the conversion of glucose to acetyl-CoA by:

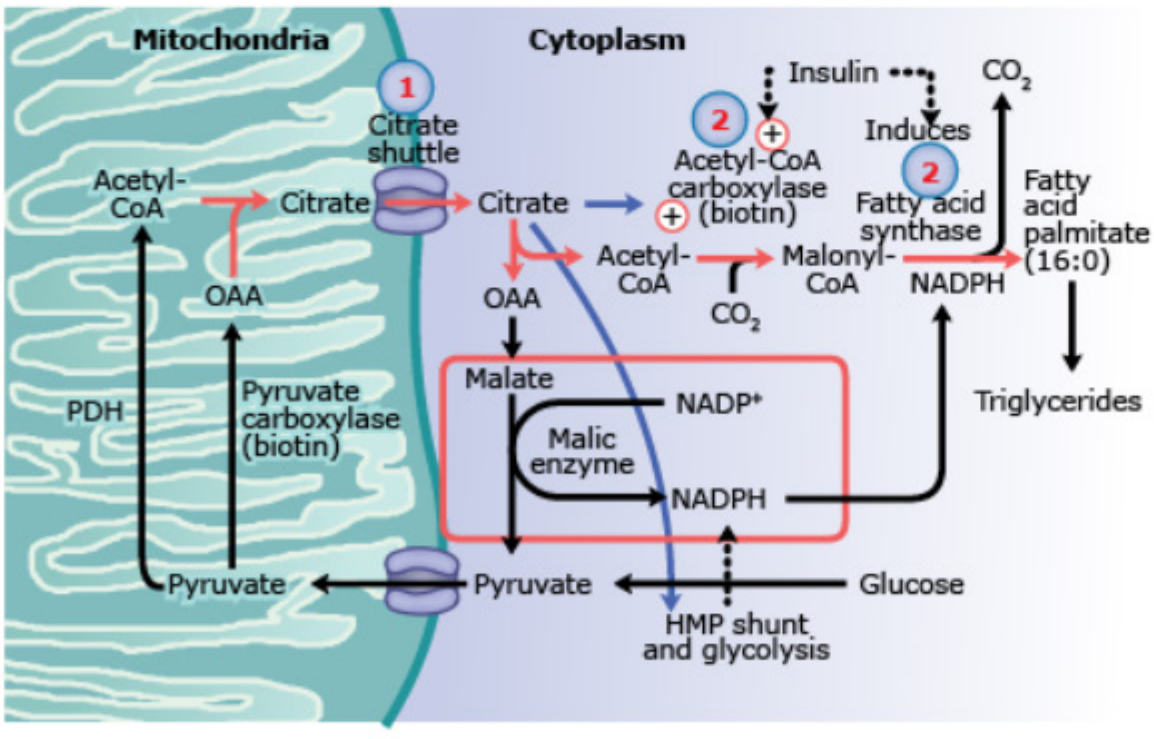
- Induction of glucokinase.
- Dephosphorylation of PFK-2/PFK-1.
- Dephosphorylation of pyruvate dehydrogenase.
- Activation of acetyl-CoA carboxylase by dephosphorylation.
- Induction of fatty acid synthase.

In addition to acetyl-CoA, the major substrates for this reaction are NADPH and ATP:

$$8 \text{ acetyl-CoA} + 14 \text{ NADPH} + 7 \text{ ATP} \rightarrow \text{palmitate (16:0)} + 14 \text{ NADP}^+ + 7 \text{ ADP} + 8 \text{ CoA}$$

3.1 Citrate Shuttle and Malic Enzyme

The transport of acetyl-CoA groups from the mitochondria to the cytoplasm is accomplished by the *citrate shuttle*. In the presence of insulin or a high-energy status, the citrate formed from acetyl-CoA combining with oxaloacetate in the mitochondria is not passed into the citric acid cycle but is instead transported to the cytoplasm. There, citrate lyase splits citrate back into acetyl-CoA and oxaloacetate, which then can be returned to the mitochondria to transport more acetyl-CoA. The reaction involves *malic enzyme* and serves as an additional source of cytoplasmic NADPH in liver and adipose tissue.



▲ **Figure 12–3.1** Synthesis of Palmitate From Glucose

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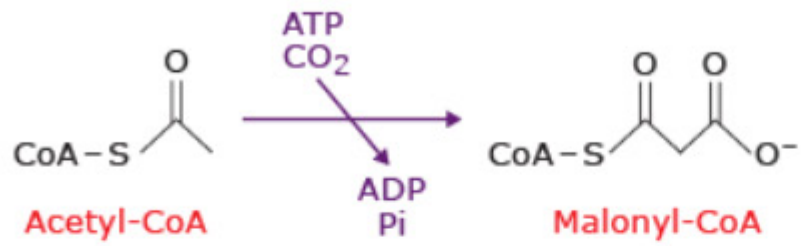
3.2 Acetyl-CoA Carboxylase

The rate-limiting enzyme of fatty acid biosynthesis is acetyl-CoA carboxylase (ACC), which activates acetyl-CoA in the cytoplasm for incorporation into fatty acids. This enzyme requires biotin as a cofactor, as well as ATP and CO_2 as substrates. This process is activated by insulin and citrate and is feedback-inhibited by malonyl-CoA and palmitoyl-CoA.



▲ **Figure 12–3.2** Reaction Catalyzed by Acetyl-CoA Carboxylase

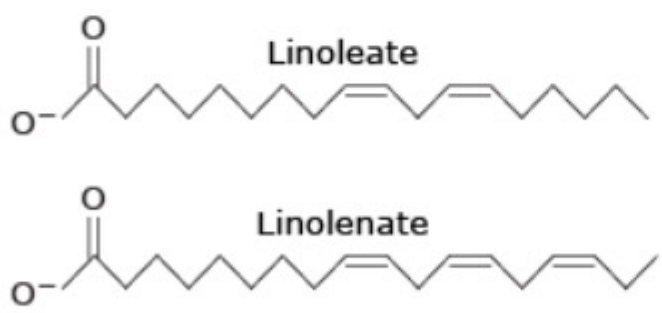
3.3 Fatty Acid Synthase

Fatty acid synthase (palmitate synthase) is a large, multienzyme complex in the cytoplasm that is induced in the liver by increased insulin levels following a meal high in carbohydrates. The enzyme synthesizes palmitate using malonyl-CoA as a substrate, but only the carbons from acetyl-CoA are actually incorporated into the fatty acid produced. Enzymes in the smooth endoplasmic reticulum may elongate fatty acyl-CoA up to C20 in increments of two carbons, and desaturate it (up to twice per fatty acid in humans) using cytochrome b_5.

3.4 Essential Fatty Acids

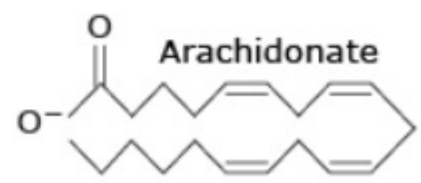
There are only two essential fatty acids, that is, fatty acids the body cannot manufacture and therefore must be absorbed from the diet. These are:

- Linoleate (18:2 [9,12])
- Linolenate acid (18:3 [9,12,15])



▲ **Figure 12–3.4A** Essential Fatty Acids

Our cells cannot make these fatty acids because they cannot make double bonds beyond carbon 9. Linoleate and linolenate are important because they serve as precursors for eicosanoids, 20-carbon structures with important physiological properties. The key eicosanoid from which all others are synthesized is arachidonate (20:4 [5,8,11,14]).



▲ **Figure 12–3.4B** Percursor of All Arachadonic Acid Metabolites

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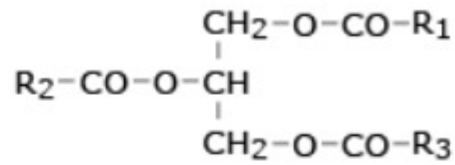
4 Triglyceride (Triacylglycerol) Synthesis

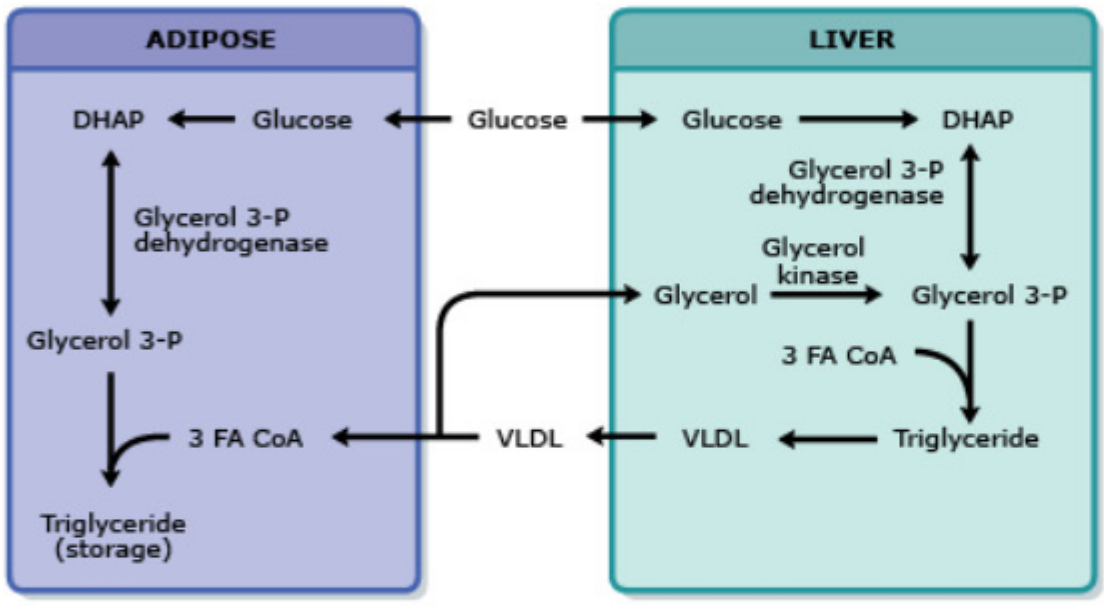
Triglycerides (TGs) are the storage form of fatty acids and are formed by attaching fatty acyl-CoA to glycerol. Triglyceride formation from fatty acids and glycerol 3-phosphate occurs in the liver and adipose tissue. The liver sends triglycerides to adipose tissue as very low-density lipoproteins (VLDL).



▲ **Figure 12–4.0** Triacylglycerol

4.1 Sources of Glycerol 3-Phosphate

Glycolytic intermediates such as dihydroxyacetone phosphate (DHAP) and recycled glycerol are the most common sources for the synthesis of glycerol 3-phosphate. In the liver, glycerol 3-P dehydrogenase reduces DHAP from glycolysis to produce glycerol 3-phosphate. The liver also may use glycerol kinase to phosphorylate free glycerol. Adipose tissue is only capable of making glycerol 3-phosphate from reduction of DHAP.



▲ **Figure 12–4.1** Source Pathways for Triglyceride Synthesis and Storage

4.2 Phospholipids

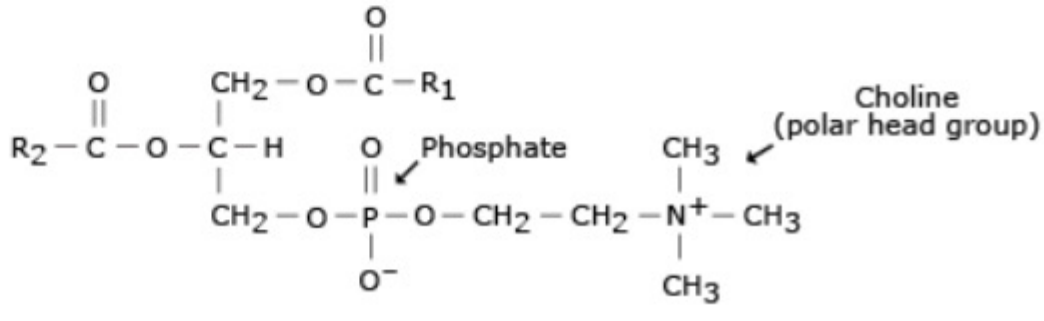
Phospholipids are structurally similar to triglycerides except instead of a fatty acid there is a phosphate attached to a polar head group on the third carbon of glycerol.

Various head groups are added to form the five major phospholipids:

- Ethanolamine: Phosphatidylethanolamine
- Choline: Phosphatidylcholine
- Serine: Phosphatidylserine
- Inositol: Phosphatidylinositol
- Glycerol: Phosphatidylglycerol

Functions of phospholipids:

- Cell membranes
- Intracellular signal transduction
- Membrane protein linkage
- Lung surfactant



▲ **Figure 12–4.2** Phosphatidylcholine

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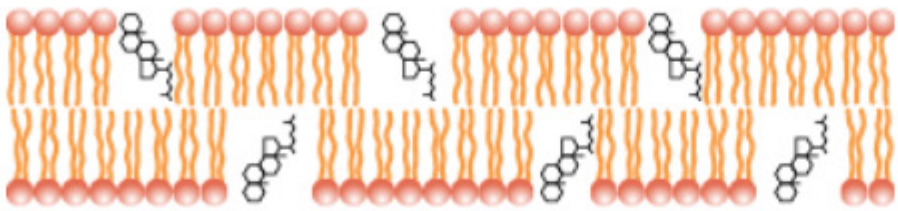
5 Cholesterol Synthesis

Cholesterol is a four-ringed lipid of 27 carbons. The hydroxyl group at carbon 3 makes cholesterol amphipathic (both hydrophilic and hydrophobic). This allows cholesterol to insert into membranes, with the hydroxyl group facing the aqueous phase and the hydrophobic tail sticking into the membrane.

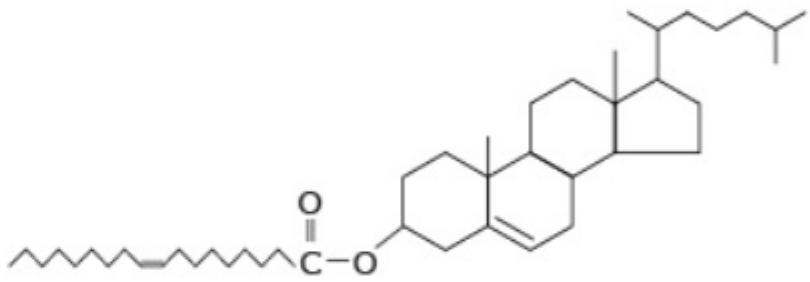


▲ **Figure 12–5.0A** Cholesterol in Phospholipid Membranes

Cholesterol has several functions. It is an essential component of eukaryotic membranes, modulating their fluidity.
It is also an important biosynthetic precursor of:

- Bile acids
- Steroid hormones
- Vitamin D

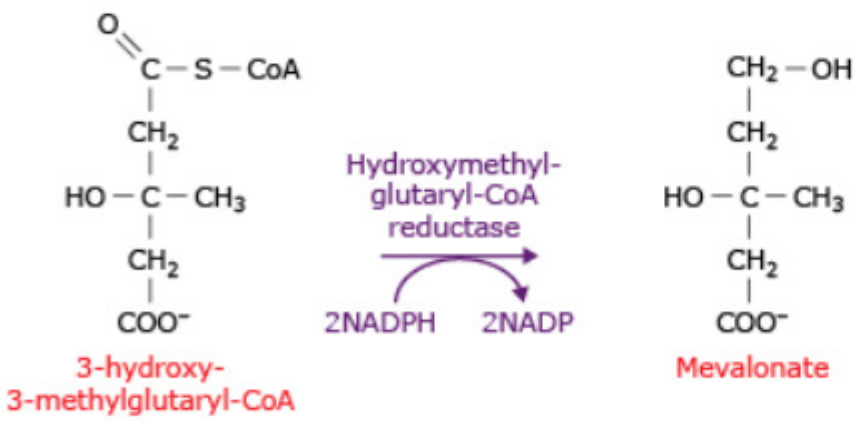
The storage form of cholesterol is cholesterol ester, a molecule of cholesterol bound by an ester linkage to the fatty acid oleate (18:1).



▲ **Figure 12–5.0B** Cholesterol Ester

5.1 De Novo Cholesterol Synthesis

Cholesterol is synthesized de novo from acetyl-CoA. This synthesis is a multistep process, the details of which you do not need to memorize. The key step is the conversion of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA; made from 3 acetyl-CoA molecules) to mevalonate.



▲ **Figure 12–5.1** Reaction Catalyzed by HMG-CoA Reductase

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This reaction is catalyzed by HMG-CoA reductase, and is the committed and rate-limiting step in cholesterol synthesis. HMG-CoA reductase is subject to negative feedback regulation at two levels:

- Increased mevalonate decreases the transcription of HMG-CoA reductase, leading to lower mRNA levels.
- Mevalonate also increases degradation of HMG-CoA reductase protein.

5.2 Smith-Lemli-Optiz (SLO) Syndrome

SLO syndrome is one of the more common autosomal recessive disorders in North American white populations (1 in 10,000 to 20,000 live births). It is characterized by multiple congenital anomalies, failure to thrive, and developmental delay or intellectual disability. The disease is caused by a mutation in 7-dehydrocholesterol-δ-7 reductase (DHCR7), which catalyzes the last step in cholesterol synthesis, conversion of 7-dehydrocholesterol to cholesterol. The consequence is the failure to synthesize adequate supplies of cholesterol to meet the needs of a developing fetus and infant.

The pathologic features may be due to deficiencies in one or more of the following:

- Steroid hormone synthesis
- Nerve axon myelination
- Processing of sonic hedgehog (SHH), a signaling protein essential for certain parts of embryogenesis

Treatments include a high-cholesterol diet and use of HMG-CoA reductase inhibitors to prevent the accumulation of 7-dehydrocholesterol, which may be involved in the pathogenesis of the disease. There is some evidence that these treatments help the symptoms of the disease; however, there is no cure.

Connection to Pharmacology

Drugs have been designed to inhibit HMG-CoA reductase, thus inhibiting de novo synthesis of cholesterol, lowering serum cholesterol, and decreasing atherosclerosis and the risk of heart attack. The classic example of these drugs (called statins) is lovastatin.

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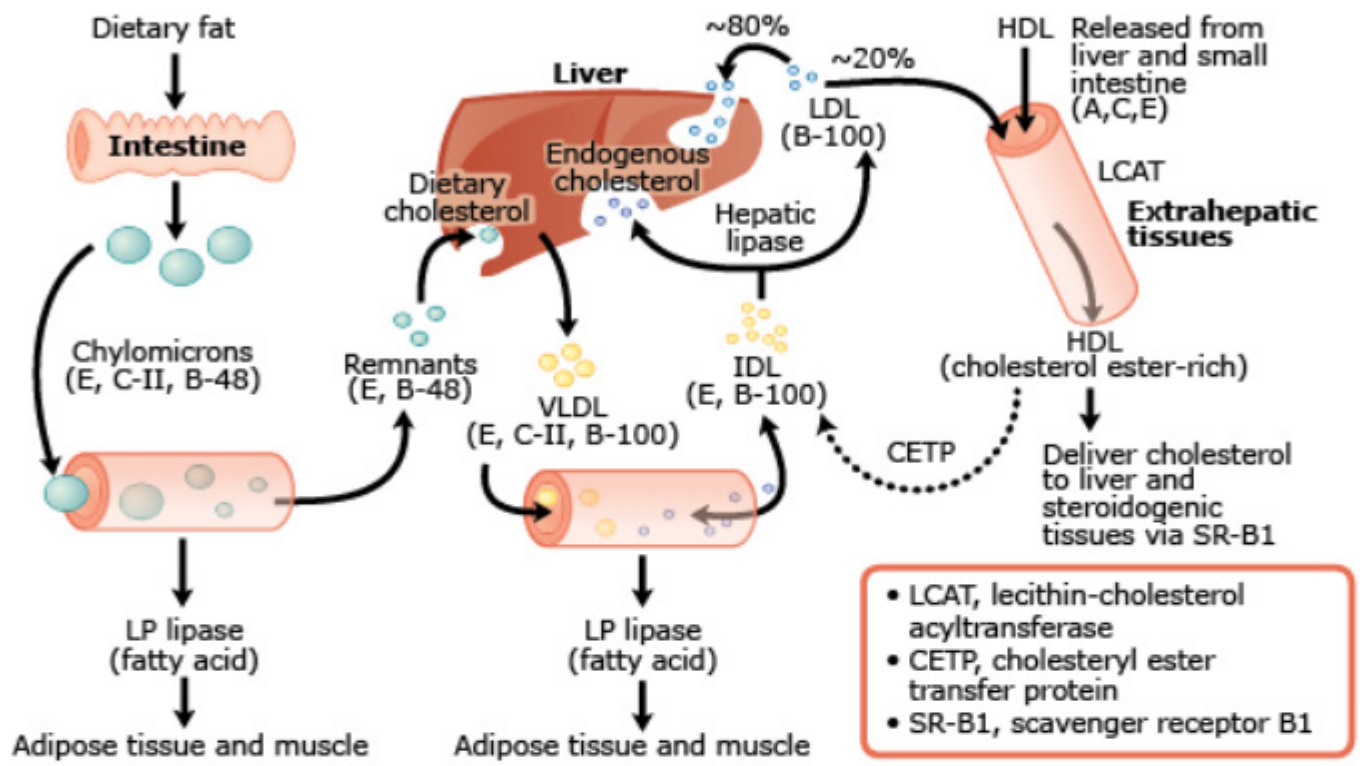
6 Lipoprotein Metabolism

Lipid digestion begins in the mouth where salivary lipase cleaves triglycerides (TGs) to diacylglycerol and free fatty acids. Upon entry into the duodenum, bile acids produced by the liver emulsify the lipid contents. As food passes into the jejunum and ileum, pancreatic production of lipase, colipase, and cholesterol esterase degrades the lipids to 2-monoglyceride, fatty acids, and cholesterol. These lipids are absorbed and re-esterified to triglycerides and cholesterol-ester.

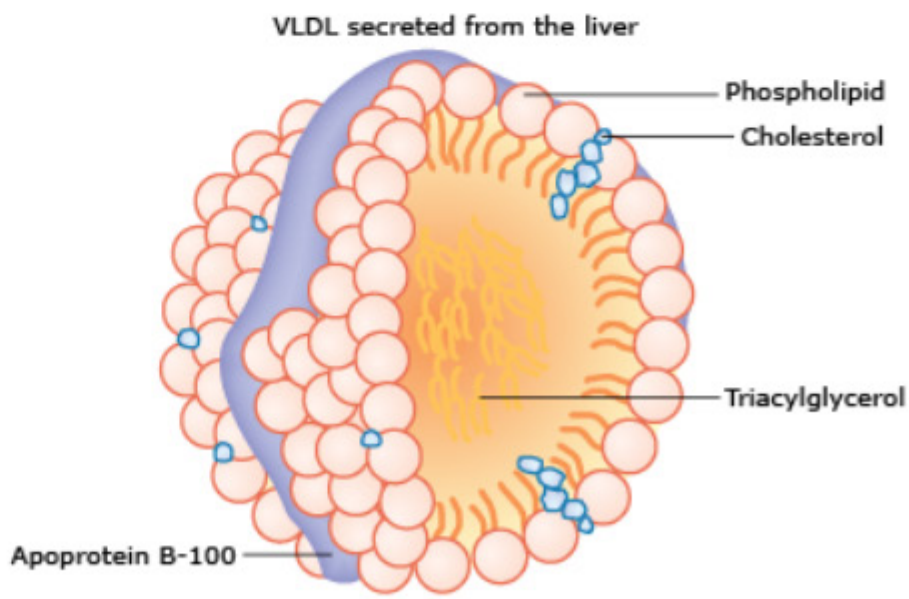


▲ **Figure 12-6.0** Lipoprotein Metabolism

6.1 Formation of Lipoproteins

Cholesterol and triglycerides are transported in the blood as *lipoproteins*. These are classified according to their density, which increases as the content of protein increases. The least dense of these lipoproteins is the chylomicron, and the most dense is the high-density lipoprotein (HDL). Lipoproteins are spherical particles consisting of:

- A surface membrane composed of phospholipids and unesterified cholesterol.
- Surface *apoproteins* (apoA, B, C, and E), unique to each lipoprotein class; these provide structure and allow the lipoprotein to be recognized by specific receptors.
- A core of triglycerides (TGs) and cholesterol esters (CEs).



▲ **Figure 12–6.1** Lipoprotein Structure

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6.2 Important Lipoproteins and Apoproteins

▼ **Table 12–6.2** Classes of Important Lipoproteins and Apoproteins

Lipoprotein	Function	Apoprotein	Function
Chylomicron	Transport of dietary triglyceride and cholesterol from intestine to tissues	apoB-48 apoC-II apoE	Activates lipoprotein lipase, secreted by intestine (liver takes up remnants)
VLDL	Transports triglycerides from liver to tissues	apoB-100 apoC-II apoE	Activates lipoprotein lipase, secreted by liver (liver takes up IDL remnants)
IDL (VLDL remnants)	Picks up cholesterol from HDL to become LDL	apoE apoB-100	Uptake by liver
LDL	Delivers cholesterol to cells	apoB-100	Uptake by liver and other tissues with LDL receptor (apoB-100 receptor)

6.2.1 Chylomicrons and VLDL

Chylomicrons and VLDL have similar functions, so they will be considered together. Chylomicrons carry lipids from the GI tract to the peripheral tissues, then to the liver. VLDL carries lipids from the liver to the peripheral tissues, then back to the liver. Both chylomicrons and VLDL deliver TGs to peripheral tissues via lipoprotein lipase (LPLase), which is attached to the capillary endothelium. LPLase is induced by insulin and activated by binding apoC on chylomicrons and VLDL. It then hydrolyzes TGs to FFAs, which are absorbed by the peripheral tissues and are either re-esterified to TGs or oxidized.

6.2.2 VLDL Remnants (IDL)

As the triglycerides are removed by LPLase, the particles become smaller and denser and the resulting particles are known as VLDL remnants or intermediate-density lipoproteins (IDL). This process is facilitated by cholesterol ester transfer protein (CETP), and also interaction with HDL. IDLs are transition particles between triglycerides and cholesterol transport. Clearance of IDL occurs by three mechanisms:

- Receptor mediated endocytosis in the liver.
- Liver LDL receptors interacting with apoB and apoE.
- Conversion to LDL by hepatic lipase, gaining cholesterol esters and losing apoE in the process.

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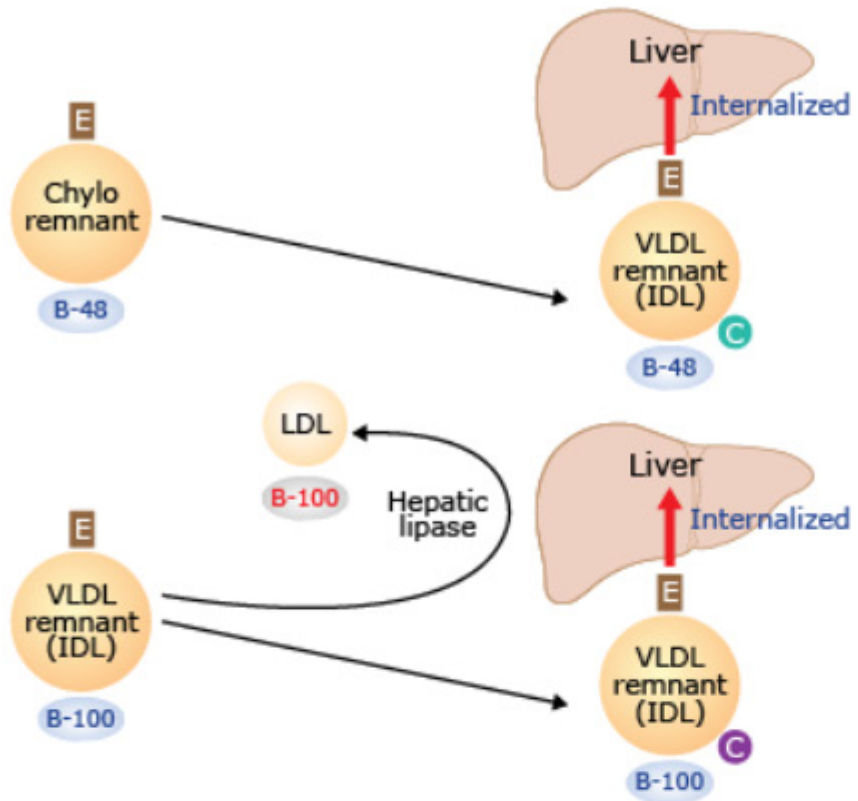
6.3 Lipid Transport



▲ **Figure 12–6.3** Transport of Chylomicrons and VLDL

6.3.1 LDL

Most of the cholesterol measured in the blood is associated with LDL. The primary role of LDL is to deliver cholesterol to tissues for membrane synthesis, steroid synthesis, and formation of bile salts in the liver. LDL does not have apoE (only apoB-100), so it is cleared much more slowly by the liver, although the liver still clears 80% of LDL.

6.3.2 HDL

The liver and intestines synthesize HDL and release it into the blood. HDL is the source of apoC and apoE for chylomicrons and VLDL, and it also contains apoA-1, which is used for the recovery of cholesterol from fatty streaks in blood vessels. The primary role of HDL is the clearance of free-tissue and plasma cholesterol to the liver.

6.3.3 Transfer of Cholesterol Between Molecules

Fatty acids are added to cholesterol in the blood by an enzyme known as *lecithin-cholesterol acyltransferase (LCAT)*. The resulting cholesterol esters dissolve readily in HDL, allowing HDL to transport cholesterol from peripheral tissues to the liver. Another protein, called *cholesterol ester transfer protein (CETP)*, transfers cholesterol esters picked up by HDL in peripheral tissues to other lipoprotein particles, such as VLDL remnants (IDL). This transfer of cholesterol transforms IDL to LDL.

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6.4 Cholesterol Regulation in Hepatocytes

There are multiple mechanisms in the liver for acquiring cholesterol, most of which use sources outside the liver. Both LDL and IDL are transported into hepatocytes by endocytosis and then fused with lysosomes to release cholesterol. A third source is HDL, which transfers cholesterol into cells by SR-B1 receptors. And finally, hepatocytes can synthesize cholesterol de novo from acetyl-CoA, catalyzed by the rate-limiting enzyme HMG-CoA reductase.

The SR-B1 receptor *("scavenger receptor")* provides a mechanism for cells to transport HDL cholesterol from the periphery into the cytoplasm. Steroidogenic tissues (gonads and adrenal glands) and hepatocytes demonstrate high levels of expression of this receptor, indicating their characteristically high demand for cholesterol. Cholesterol transport by this receptor does not include endocytosis of HDL, but the precise mechanism of transport is not well understood.

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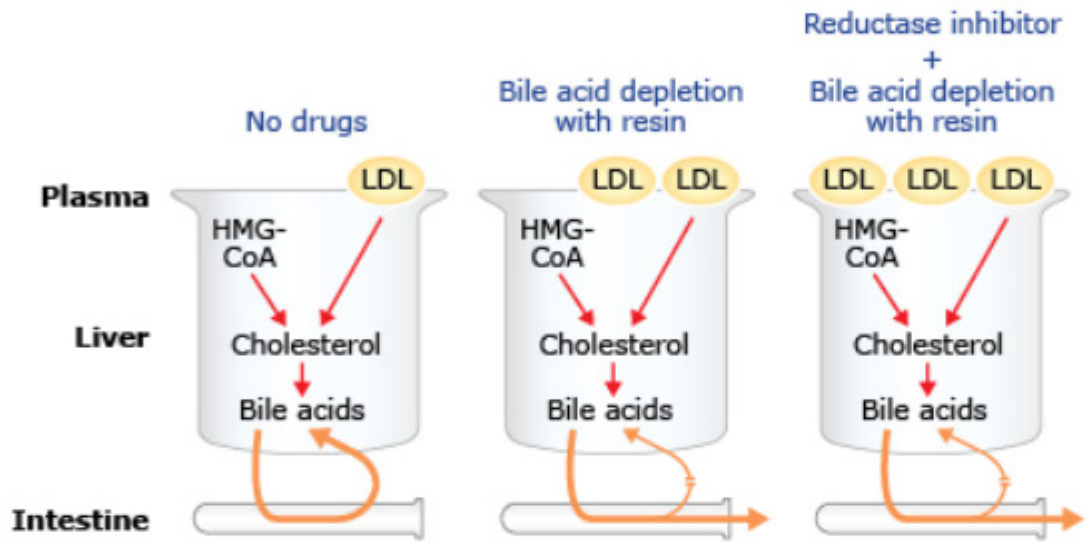
7 Hyperlipidemias

High plasma LDL can develop in those who consume a high-fat diet. In these individuals, plentiful cholesterol supply causes a down-regulation of the LDL receptor, increasing the plasma LDL with the same consequences as those in the genetic disease. Treatment includes cholesterol-binding resins (cholestyramine, colestipol) and HMG-CoA reductase inhibitors (statins).



▲ **Figure 12–7.0** Treatment of Hypercholesterolemia

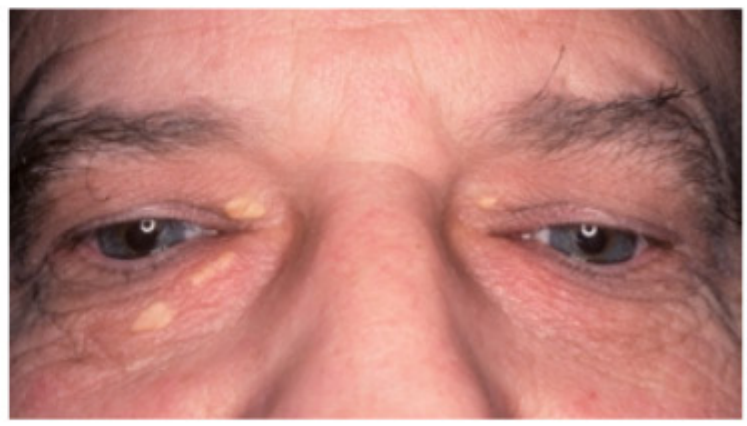
7.1 Type I: Hypertriglyceridemia

Also called type 1 hyperlipoproteinemia, this disease is caused by a genetic deficiency of lipoprotein lipase or apoC. Patients are unable to clear chylomicrons or VLDL from serum, leading to marked elevation of triglycerides, which, in turn, leads to elevated chylomicrons in the blood. Clinical symptoms include recurrent pancreatitis and xanthomas.

7.2 Type IIa: Hypercholesterolemia (LDL Receptor Deficiency)

Type IIa, or familial hypercholesterolemia (FH), is caused by mutations in the LDL receptor. The consequence of these mutations is that LDL is not cleared from the circulation. Circulating LDL has a propensity for being oxidized. Once oxidized, it cannot be cleared by LDL receptors. Instead, it is scavenged by subendothelial macrophages. This process causes atherogenesis, endothelial damage, and clot formation. Cholesterol deposits also may be seen as:

- Xanthomas of the Achilles tendon.
- Subcutaneous tuberous xanthomas over the elbows and knees.
- Xanthelasma (lipid in the eyelid).
- Corneal arcus.



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▲ **Figure 12-7.2A** Xanthelasmas

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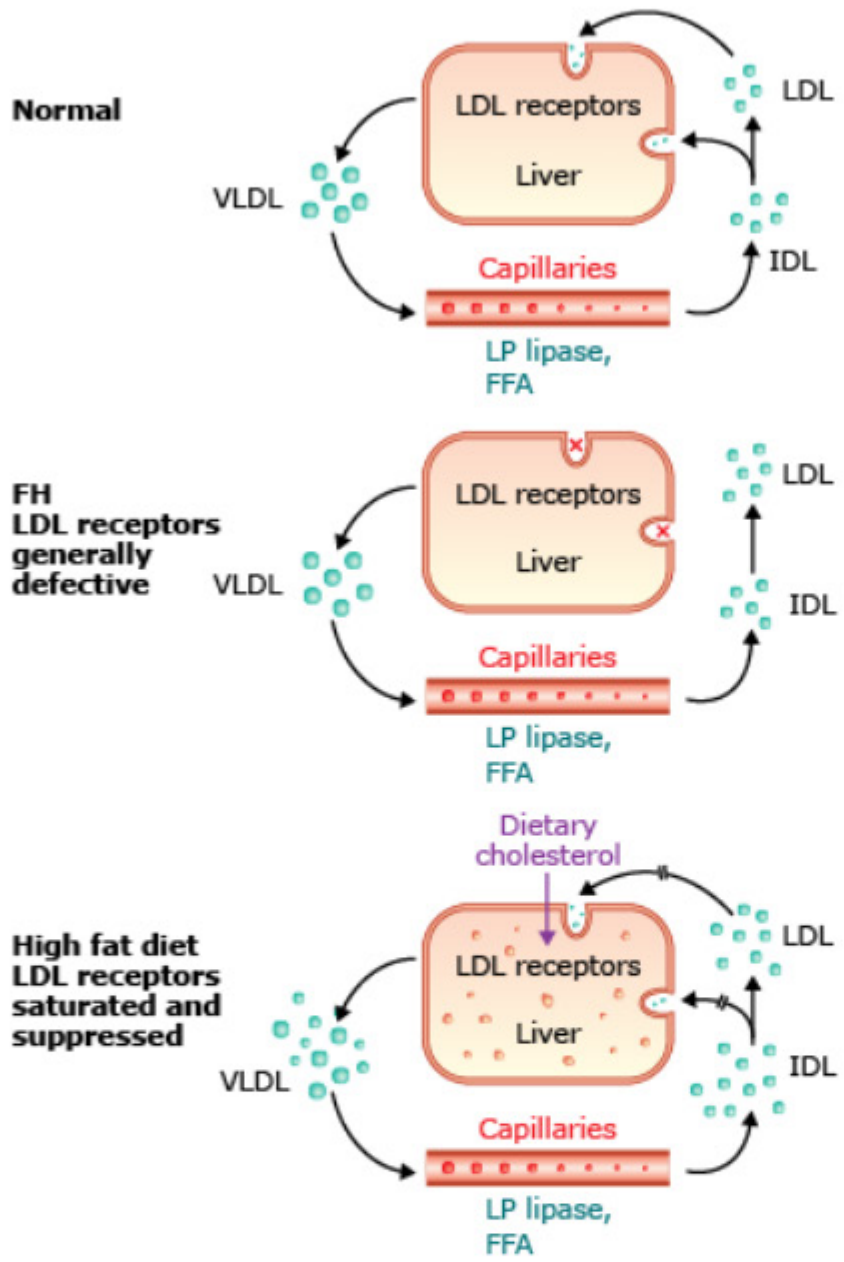
FH is the most common Mendelian disorder, with a heterozygote frequency of ~1/500. Heterozygotes have a two- to three-fold elevation in plasma cholesterol and suffer severe atherosclerosis, often leading to MI in their 30s to 40s. Homozygotes have a five- to six-fold elevation in plasma cholesterol and develop atherosclerosis at a much earlier age, often having MIs as early as childhood.



▲ **Figure 12–7.2B** Dietary and Familial Hypercholesterolemia

7.3 Abetalipoproteinemia

Low (hypobetalipoproteinemia) and absent (abetalipoproteinemia) serum apoB-100 and apoB-48 cause serum triglycerides to be near zero and serum cholesterol to be extremely low. Low chylomicron levels cause fat to accumulate in intestinal enterocytes and in hepatocytes. Additionally, fat-soluble vitamins (A and E) as well as essential fatty acids are not well-absorbed. Symptoms include:

- Fatty diarrhea (steatorrhea)
- Degeneration of pigments in the retina
- Cerebellar ataxia
- Acanthocytes
- Possible loss of night vision

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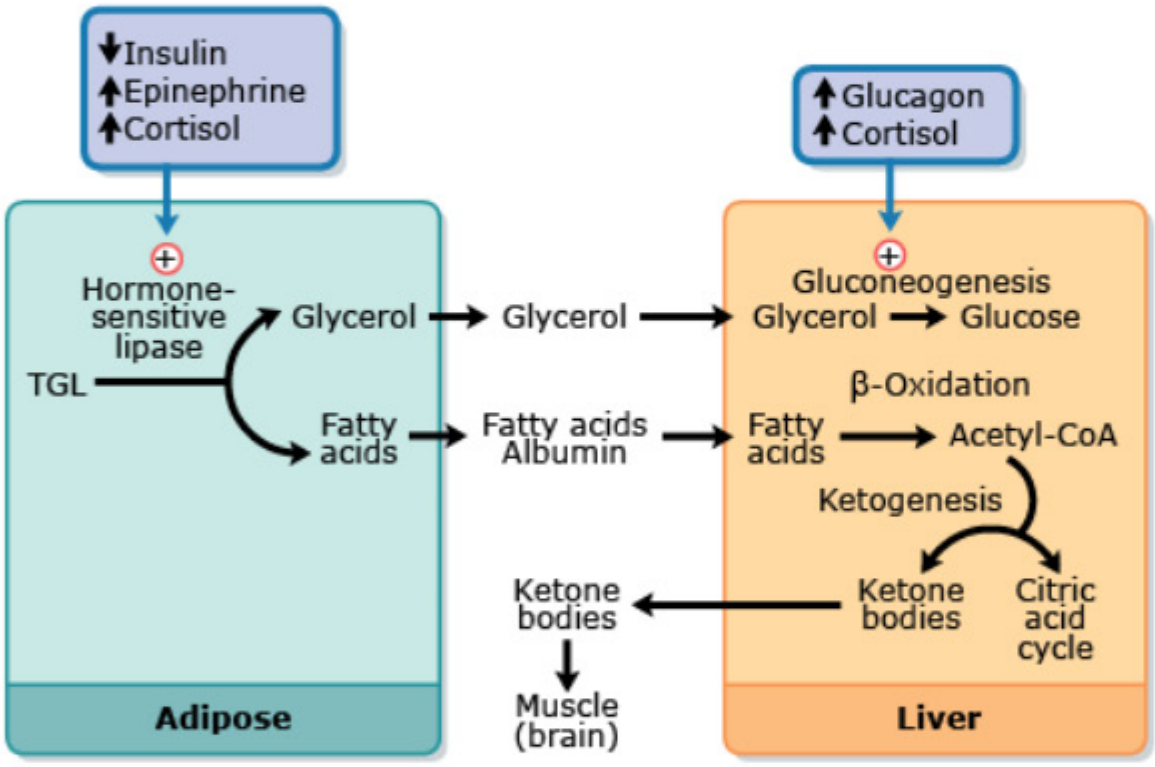
1 Lipid Mobilization

The mobilization of fatty acids from adipose tissue in the post-absorptive state occurs when a fall in insulin activates a hormone-sensitive triacylglycerol lipase (HSL). Triglycerides are thus hydrolyzed to fatty acids and glycerol. Other hormonal regulators of HSL include epinephrine and cortisol. The glycerol that is produced is converted to dihydroxyacetone phosphate (DHAP) for gluconeogenesis in the liver, and the fatty acids are distributed to tissues in association with serum albumin.



▲ **Figure 13–1.0** Lipolysis of Triglycerides in Response to Hypoglycemia and Stress

USMLE® Key Concepts

For Step 1, you must be able to:

- Describe the mobilization and regulatory control of fatty acids.
- Explain the biochemical basis of medium chain acyl-CoA dehydrogenase and myopathic carnitine deficiency.
- Identify the mechanism of the odd-carbon fatty acid oxidation through the propionic acid pathway.
- Describe the oxidation of ketone bodies and their tissue-specific usage for energy.
- Explain the biochemical mechanisms underlying the sphingolipidoses: Tay-Sachs, Gaucher, Niemann-Pick, and Fabry diseases.

Connection to Pharmacology

Niacin acts as an anti-hyperlipidemic drug in large doses. It works by inhibiting HSL in adipose tissue and thereby diminishing the entry of fatty acids into the liver. This means that very low-density lipoprotein (VLDL) will be made in smaller amounts, and its product, LDL, will be lower in serum.

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2 Fatty Acid Oxidation

Fatty acids are converted to acetyl-CoA in the mitochondria in a process known as β-oxidation. This occurs in liver, muscle, and adipose tissue. Neither erythrocytes (which lack mitochondria) nor brain cells (fatty acids do not easily cross the blood-brain barrier) can use fatty acids for energy, so these cells depend on glucose even during periods of fasting. This pathway involves three major steps:

1. Activation of free fatty acids
2. Transport into mitochondria
3. Oxidation

2.1 Activation

Fatty acids greater than two carbons long must be converted to their acyl-CoA form in order to be transported and oxidized. This is accomplished by fatty acyl-CoA synthetase on the outer mitochondrial membrane. Shorter fatty acids pass directly into the mitochondria and are activated in the matrix.

2.2 Transport

Fatty acyl-CoAs cannot cross the inner mitochondrial membrane. They first must be modified by a molecule of carnitine. This is catalyzed by carnitine palmitoyltransferase 1 (CPT 1, also known as carnitine acyltransferase-1), an enzyme in the outer mitochondrial membrane. Fatty acyl carnitine is then shuttled across the inner mitochondrial membrane, and carnitine acyltransferase-2 (also known as carnitine palmitoyltransferase-2) transfers the fatty acyl group back to a CoA in the mitochondrial matrix.

2.3 Oxidation

β-oxidation removes acetyl-CoA groups one at a time from acyl-CoA and thereby reverses the process of fatty acid synthesis. There are four enzymatic reactions required for each oxidation cycle. In the process, one $FADH_2$ and one NADH are produced in addition to acetyl-CoA. $FADH_2$ and NADH are then oxidized in the electron transport chain to provide ATP. In adipose and muscle tissue, the acetyl-CoA is run through the citric acid cycle. In the liver, gluconeogenesis can be accomplished using the ATP generated by the citric acid cycle, and the acetyl-CoA further stimulates the process by activating pyruvate carboxylase.

In the fasting state, the liver produces more acetyl-CoA than is used in the citric acid cycle. This excess acetyl-CoA is then used to produce ketone bodies that are released into the blood for use in other tissues.

Connection to Physiology

The presence of malonyl-CoA from fatty acid synthesis inhibits carnitine acyltransferase-1 and thereby prevents newly synthesized fatty acids from entering the mitochondria. Insulin indirectly inhibits β-oxidation by activating acetyl-CoA carboxylase and increasing the malonyl-CoA concentration in the cytoplasm. Glucagon counteracts this process.

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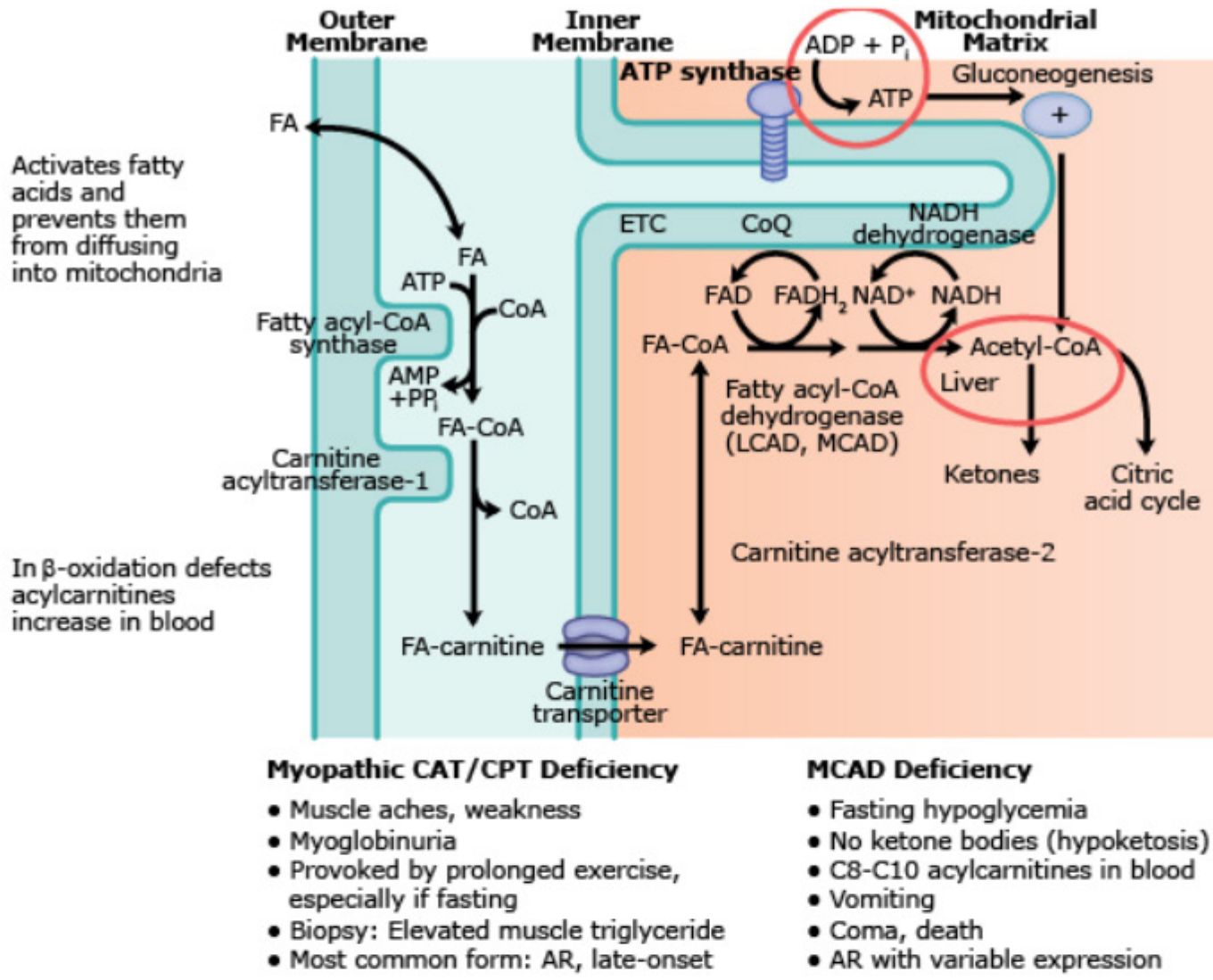

▲ **Figure 13–2.3** Fatty Acid Activation, Transport, and β-Oxidation

2.4 Genetic Deficiencies of Fatty Acid Oxidation

2.4.1 Medium Chain Acyl-CoA Dehydrogenase Deficiency

Medium-chain acyl-CoA dehydrogenase (MCAD) catalyzes the first step in the oxidation cycle for fatty acids of 6 to 12 carbons in length. When it is deficient, patients cannot mobilize fatty acids for energy during periods of relative starvation. Consequently, symptoms tend to appear as infants begin to sleep for longer periods at night. With severely decreased fatty acid oxidation, the tissues are more reliant on glucose for energy and a profound hypoglycemia results. Normally, hypoglycemia would be accompanied by ketosis, but without fatty acid oxidation, ketone bodies cannot be produced. Thus, MCAD deficiency results in a hypoketotic hypoglycemia. Signs and symptoms of MCAD deficiency include:

- Hypoglycemia
- Vomiting
- Lethargy
- Hepatomegaly (from accumulation of medium-chain fatty acids)
- Encephalopathy
- Seizures
- Cardiopulmonary arrest
- Sudden death

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Homozygous MCAD deficiency has been linked to a certain fraction of sudden infant death syndrome cases. The incidence of MCAD deficiency is believed to be 1/10,000, which makes it one of the most common of the inborn errors of metabolism. Treatment includes frequent high-carbohydrate meals and carnitine to increase fatty acid oxidation. Long-term complications—including neurologic, developmental, and behavioral problems—have been seen even in treated children.

2.4.2 Myopathic Carnitine Acyltransferase-2 Deficiency

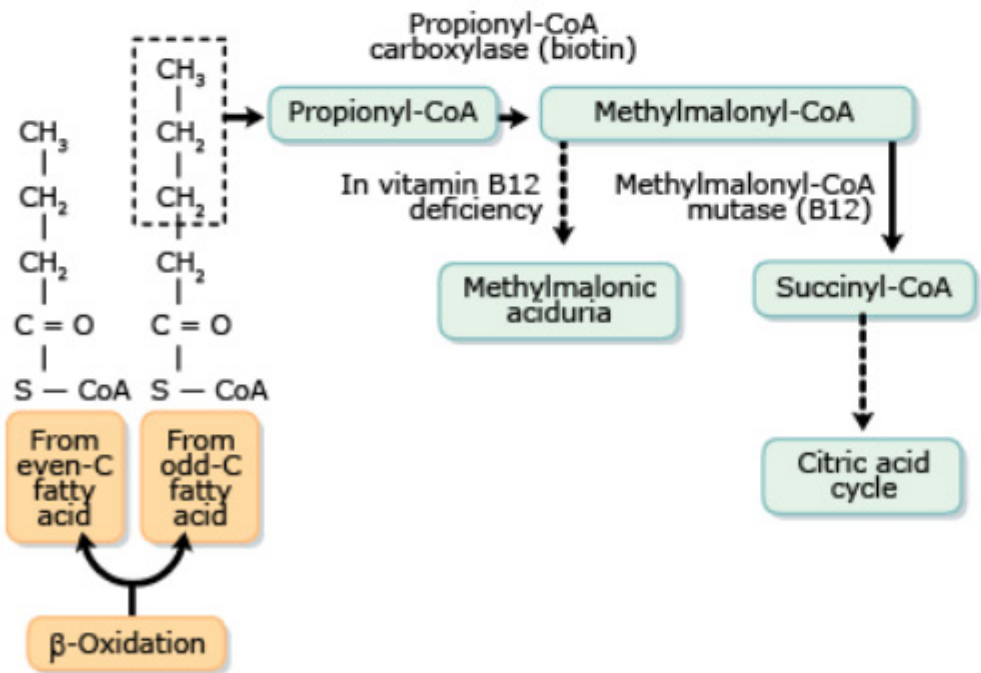
A defect in the muscle-specific carnitine acyltransferase-2 (CAT/CPT) produces an adolescent or adult onset syndrome characterized by muscle aches and weakness, rhabdomyolysis, and myoglobinuria. These episodes are provoked by prolonged exercise or stress, and are exacerbated by high-fat, low-carbohydrate diets. The diagnosis is made by finding lipid droplets in the cytoplasm of muscle cells on biopsy, and treatment involves therapy with glucose.

2.5 Propionic Acid Pathway

When fatty acids with odd numbers of carbon atoms are β-oxidized, the process is identical up to the point of the final cycle. Now the odd-carbon fatty acids produce one acetyl-CoA and one propionyl-CoA from the five-carbon fragment remaining. The two-step propionic acid pathway converts propionyl-CoA to succinyl-CoA, which can then form malate and promote gluconeogenesis. This means that odd-carbon fatty acids are the only exception to the rule that fatty acids cannot be converted to glucose in humans.

The mitochondrial enzymes involved in the propionic acid pathway are:

- Propionyl-CoA carboxylase (requires biotin)
- Methylmalonyl-CoA mutase (requires vitamin B12)



▲ **Figure 13–2.5** Propionic Acid Pathway

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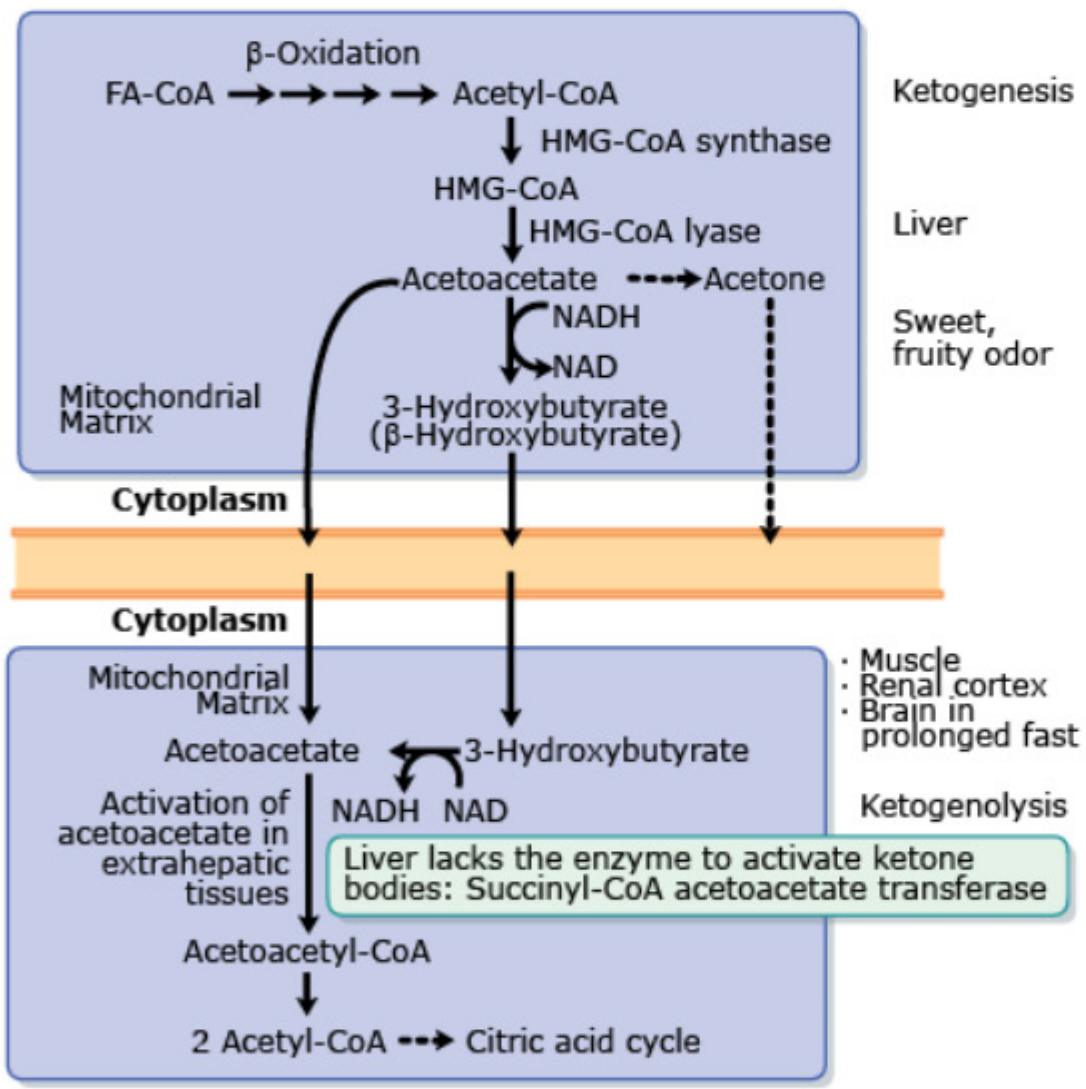
3 Ketone Body Metabolism

3.1 Ketogenesis

In the fasting state, the liver converts acetyl-CoA from the β-oxidation of fatty acids into acetoacetate, β-hydroxybutyrate, and acetone (ketone bodies). Cardiac and skeletal muscles and the renal cortex metabolize acetoacetate and β-hydroxybutyrate to acetyl-CoA. Normally, muscle metabolizes ketones as quickly as the liver produces them, but in periods of prolonged fasting, ketone bodies are especially important for the brain. Fatty acids cannot be transported across the blood-brain barrier. Consequently, the brain is highly reliant on glucose for energy. The brain can utilize ketone bodies. Therefore, the activation of ketogenesis during hypoglycemia is crucial to the maintenance of nervous system function.



▲ **Figure 13–3.1** Ketogenesis (Liver) and Ketogenolysis (Extrahepatic)

3.2 Ketogenolysis

Ketogenolysis occurs in extrahepatic sites because the liver lacks the enzyme succinyl-CoA acetoacetyl-CoA transferase (thiophorase), which is necessary to activate acetoacetate. In the brain, the first 12 hours of fasting are managed with glucose derived from liver glycogenolysis. Beyond this point, glucose from gluconeogenesis becomes the most important fuel. After a week, the fuel changes again to use ketones derived from fatty acids.

Clinical Application

Acetone is a minor side product of ketogenesis that is not used as fuel in tissues. It imparts a strong sweet or fruity odor to the breath, however, which is pathognomonic for ketoacidosis.

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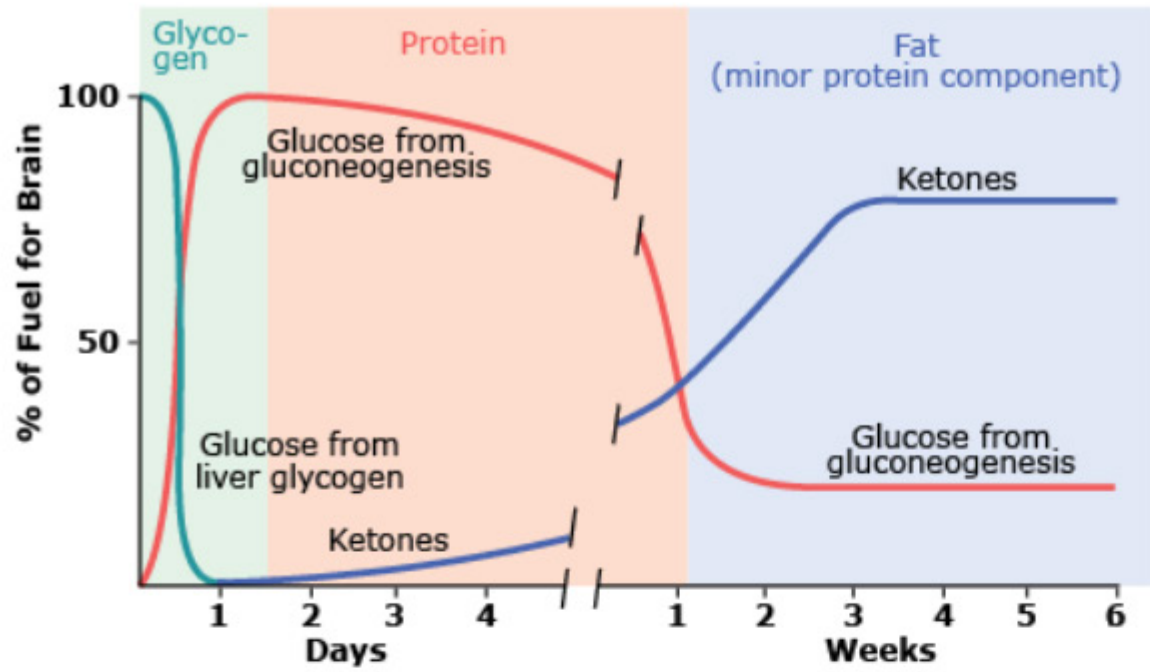

▲ **Figure 13–3.2** Fuel Use in Brain During Fasting and Starvation

3.3 Ketoacidosis

In uncontrolled type 1 insulin-dependent diabetes mellitus, the release of fatty acids from adipose tissue and ketone bodies from the liver exceed the ability of the body to metabolize them. This can result in a life-threatening ketoacidosis. In type 2 non-insulin-dependent diabetes mellitus, ketoacidosis is much less common, although the basis for this observation is unclear. Alcoholics also are prone to ketoacidosis due to chronic hypoglycemia, which causes fat release from adipose tissue. Ketone production by the liver is increased, but muscle use is slower because alcohol is converted to acetate in the liver and is oxidized by muscle as an alternative source of acetyl-CoA. The signs of ketoacidosis include:

- Acetone on breath
- CNS depression and coma
- Decreased plasma bicarbonate
- Depletion of K^+ (may be masked by mild hyperkalemia)
- Polydipsia, polyuria, polyphagia (exacerbated by hyperglycemia and osmotic diuresis)

In normal fasting, ketosis acetoacetate and β-hydroxybutyrate are formed in approximately equal quantities. In diabetic and alcoholic ketoacidosis, the ratio between these ketone bodies shifts and β-hydroxybutyrate will predominate. If a urinary nitroprusside test is used in these cases, it can underestimate the extent of ketoacidosis, because it measures only acetoacetate. Therefore, measurement of both blood glucose and β-hydroxybutyrate is undertaken in these patients.

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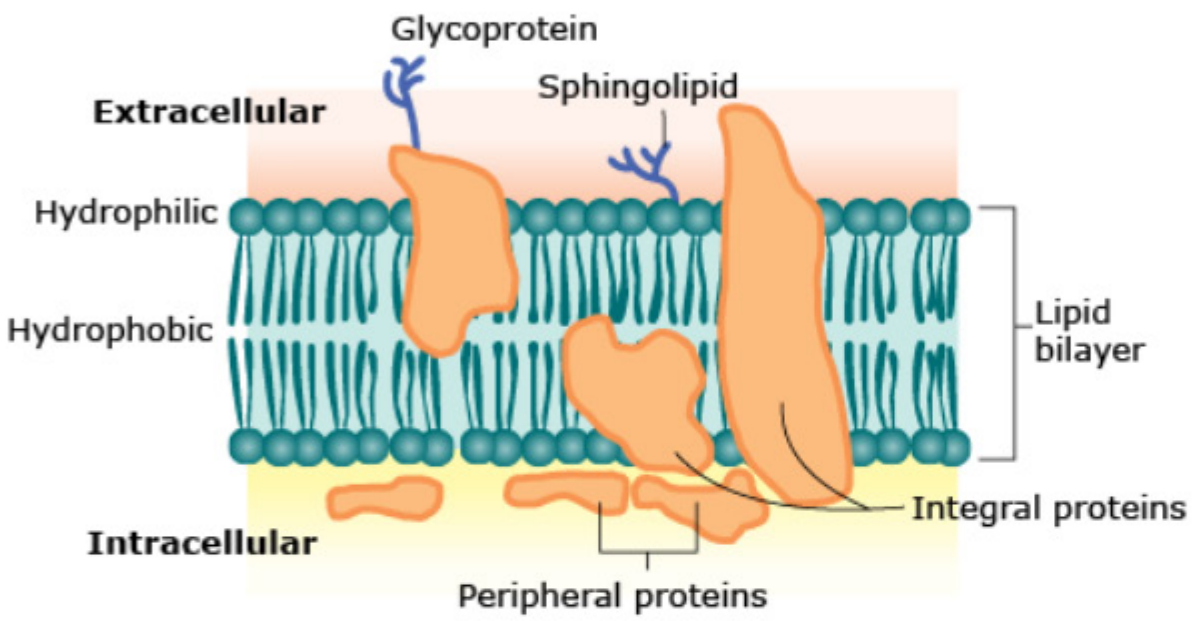
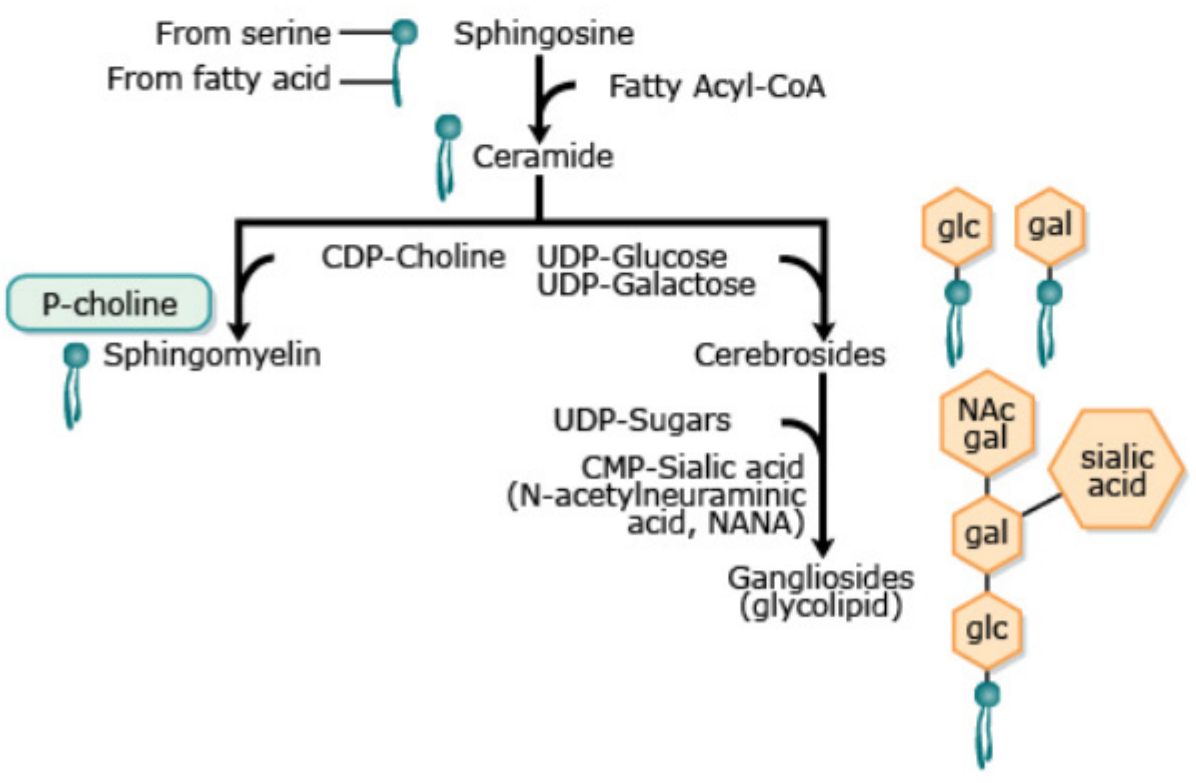
4 Sphingolipids

Sphingolipids are important components of cellular membranes. They are particularly enriched in nerve tissue. They have a structure similar to phospholipids, except that they are built on a molecule of serine, rather than glycerol. They have a hydrophilic region and two fatty-acid-derived hydrophobic tails. Classes of sphingolipids are distinguished by their hydrophilic groups as follows:

- **Cerebrosides:** Galactose or glucose
- **Gangliosides:** Branched oligosaccharide chains terminating in sialic acid
- **Sphingomyelin:** Phosphorylcholine



▲ **Figure 13–4.0A** Plasma Membrane



▲ **Figure 13–4.0B** Synthesis of Sphingolipids

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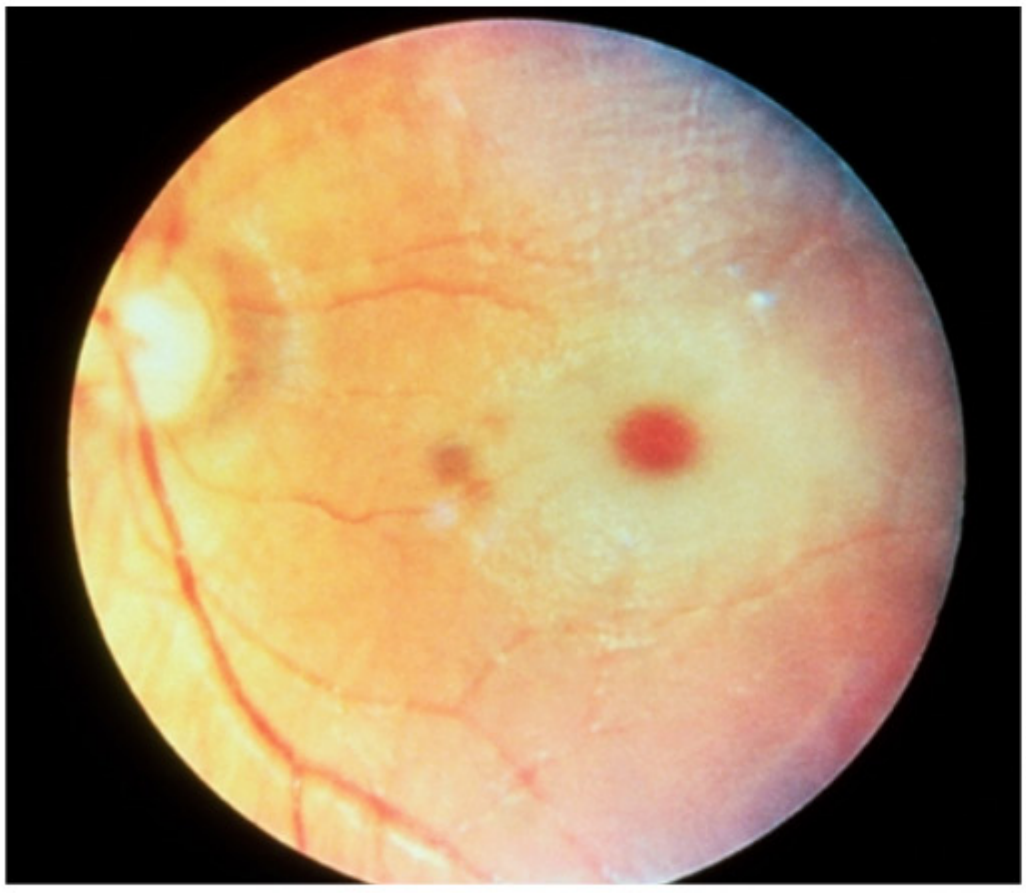
4.1 Genetic Deficiencies of Enzymes in Sphingolipid Catabolism

Sphingolipids released during membrane degradation are digested in lysosomes by sequential cleavage of sugar units from the oligosaccharide head group. A variety of enzymes are present in lysosomes to achieve this goal, and their deficiencies associate with genetic disease.

4.1.1 Tay-Sachs Disease

Tay-Sachs disease is an autosomal recessive disorder caused by mutations in the α subunit of hexosaminidase, an enzyme responsible for degrading GM_2 gangliosides. The consequence of this defect is that undegraded gangliosides accumulate in the lysosomes of cells, particularly neurons, eventually leading to cell death. This creates characteristic whorled inclusions visible by electron microscopy. The initial symptoms of Tay-Sachs disease appear at 3 to 10 months after birth and include decreased alertness, excessive sensitivity to noise (hyperacusis), and developmental delay. The classic physical exam finding early in the disease is the cherry-red spot on an otherwise pale macula. The course of the disease is progressive, leading to seizures, paralysis, blindness, and dementia. Death usually occurs by age 4.



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▲ **Figure 13–4.1** Tay-Sachs Disease

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4.1.2 Gaucher Disease

Gaucher disease results from an autosomal recessive deficiency of glucocerebrosidase, which causes the accumulation of glucocerebroside. It may present as an adult hepatosplenomegaly with erosion of bones and resultant fractures. Pancytopenia and thrombocytopenia may result, and macrophages have characteristic "crumpled paper" inclusions. Enzyme replacement therapy with recombinant glucocerebrosidase is effective but often prohibitively expensive.

4.1.3 Niemann-Pick Disease

Niemann-Pick disease results from an autosomal recessive deficiency of sphingomyelinase, and accumulation of sphingomyelin. It is characterized by hepatosplenomegaly, microcephaly, and severe intellectual disability. Cherry spots in the macula may be present. Macrophages contain "foamy" lipid-filled vesicles. Affected children die early.

4.1.4 Fabry Disease

Fabry disease results from an X-linked recessive deficiency of α-galactosidase. As a result, ceramide trihexoside accumulates in the lysosomes. The disease presents in childhood or adolescence with burning sensations in the hands, angiokeratomas on the skin, cloudiness of the cornea, impaired arterial circulation, increased risk of heart attack or stroke, and enlargement of the heart and kidneys. Renal failure is the frequent cause of death. Enzyme replacement therapy is available, and although expensive, slows the development of the disease.

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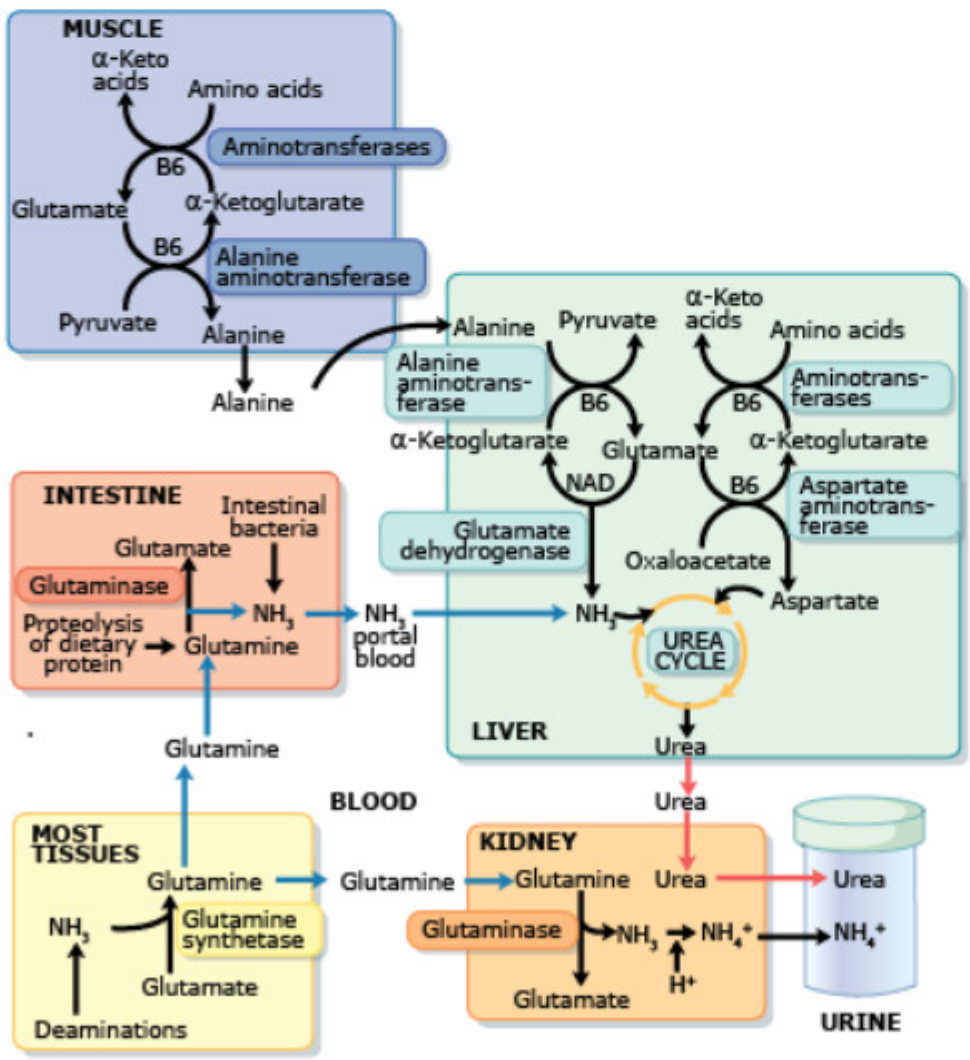
1 Overview of Nitrogen Metabolism

When protein from the diet or from muscle is used as an energy source, the amino group is removed by transamination or deamination. The carbon skeletons are then converted to glucose (glucogenic amino acids) or acetyl-CoA and ketone bodies (ketogenic amino acids). In some cases, amino acids may be both glucogenic and ketogenic.

2 Removal and Excretion of Amino Groups

Most excess nitrogen is converted into urea in the liver and then carried via the blood to the kidney, where it is released in urine. Amino groups released by deamination form ammonium ions (NH_4^+), which cannot be carried in the blood due to their potential toxicity, so most tissues transport excess nitrogen as glutamine. Muscle sends excess nitrogen to the liver as alanine or other amino acids in addition to glutamine.



▲ **Figure 14–2.0** Excretion of Amino Group as Urea and Ammonium

USMLE® Key Concepts

For Step 1, you must be able to:

- Describe the organ-specific mechanisms of amino acid deamination and their contribution to the urea cycle.
- Explain the consequences of carbamoyl phosphate synthetase and ornithine transcarbamoylase deficiency on the urea cycle.
- Identify the biochemical mechanism of disease for phenylketonuria, alcaptonuria, maple syrup urine disease, and homocystinuria.
- Describe the mechanisms of heme synthesis and bilirubin metabolism.
- Explain the biochemical bases of the porphyrias and jaundice.

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2.1 Glutamine Synthetase

In most tissues, excess nitrogen is captured by amination of glutamate to form glutamine. The enzyme glutamine synthetase performs this reaction irreversibly. Glutamine is the most common carrier of excess nitrogen in the body, and is relatively nontoxic.

2.2 Glutaminase

Once in the kidney, arriving glutamine is deaminated with kidney glutaminase and the amino group is eliminated as an ammonium ion in the urine. The reaction is irreversible. Glutaminase in the kidney is induced by chronic acidosis, and in such cases excretion of ammonium can become the major defensive mechanism. Levels of glutaminase are also high in the intestine, where the ammonium from dietary protein and intestinal bacteria can be sent directly to the liver via the portal blood to be used for synthesis of urea. The liver itself has low levels of glutaminase.

2.3 Aminotransferases (Transaminases)

In muscle and liver, aminotransferases transfer the amino group from the carbon skeleton of the amino acid to a citric acid cycle intermediate, most commonly α-ketoglutarate. Pyridoxal phosphate, derived from vitamin B6, is required for the transfer. These enzymes are named according to the amino acid that donates the amino group to α-ketoglutarate, and the most important examples are alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

The aminotransferases catalyze important reversible reactions:

- During muscle protein catabolism, aminotransferases move amino groups from a variety of amino acids to pyruvate, forming alanine, which is used to transport amino groups.
- In the liver, muscle-mobilized alanine can serve as the amino group source for aspartate, which transports the amino group into the urea cycle for subsequent elimination in the urine.

2.4 Glutamate Dehydrogenase

Glutamate dehydrogenase is found in many tissues and catalyzes the reversible oxidative deamination of glutamate. The product of this reaction is the citric acid cycle intermediate α-ketoglutarate, so this is the point of entry for several of the glucogenic amino acids into the citric acid cycle.

When ALT and AST leak into the blood from their sources in the liver and in muscle, they become useful clinical indicators of damage to those organs.

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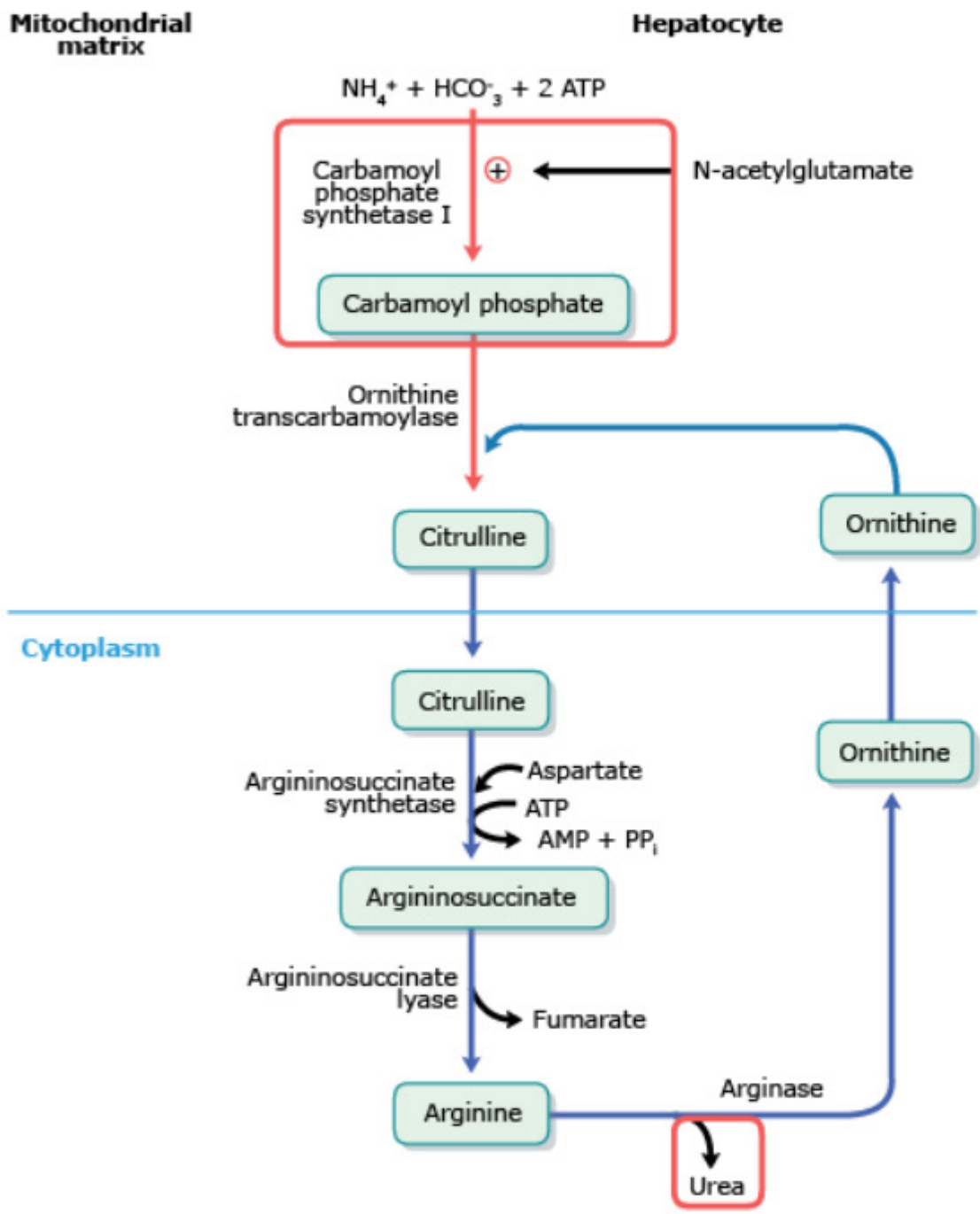
3 The Urea Cycle

In the liver, the mitochondrial enzyme carbamoyl phosphate synthetase produces carbamoyl phosphate from ammonium and carbon dioxide. N-acetylglutamate is required as an activator, and is present only when free amino acids are present. Aspartate enters the cycle in the cytoplasm, and the urea product enters the blood to be delivered to the kidney.



▲ **Figure 14–3.0** Urea Cycle in the Liver

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3.1 Genetic Defects of the Urea Cycle

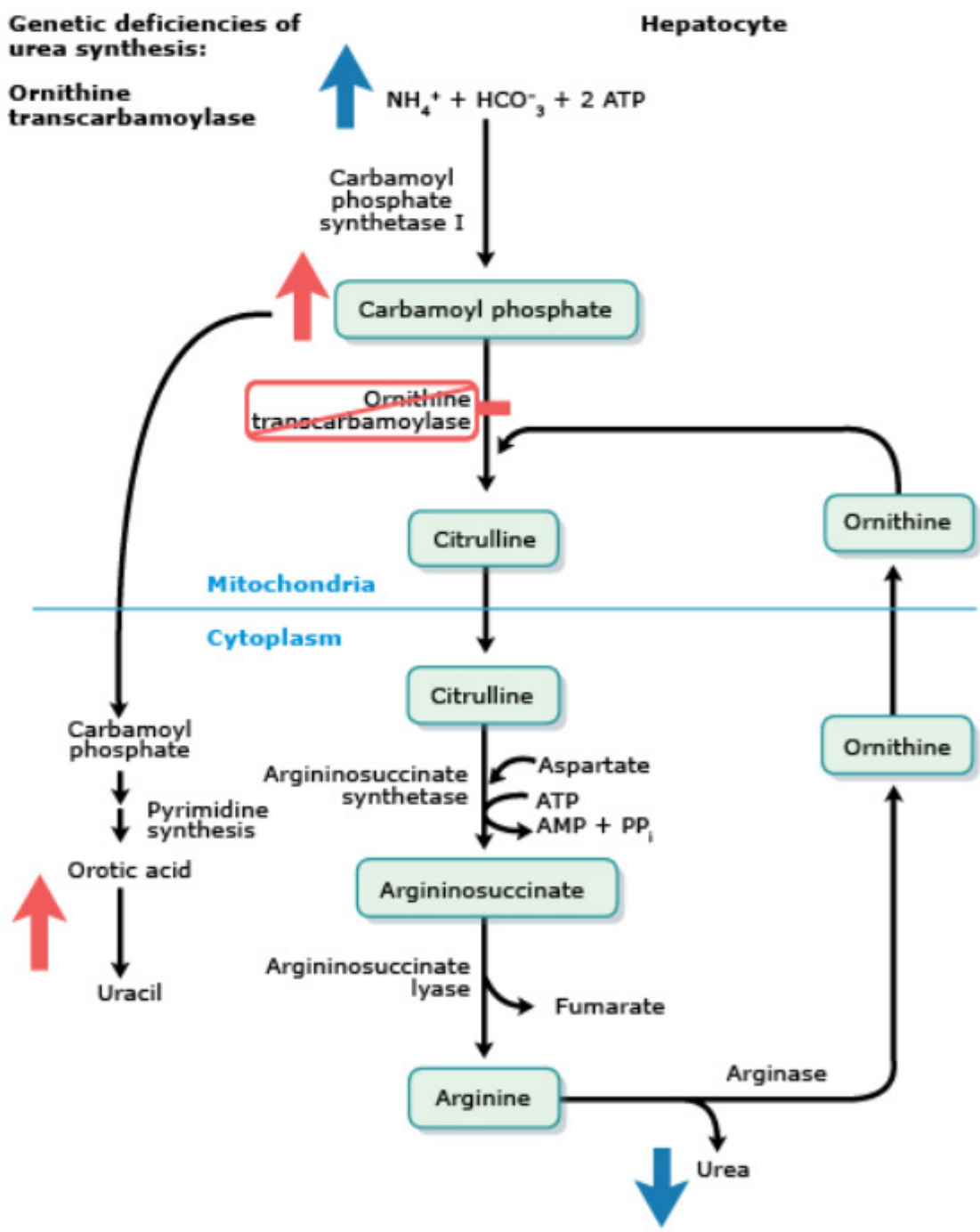
3.1.1 Ornithine Transcarbamoylase (OTC) Deficiency

OTC deficiency is an X-linked recessive disorder caused by a deficiency in the second step of the urea cycle. This is the most common of a group of diseases known as urea cycle defects (UCDs). The most damaging consequence of this defect is the accumulation of ammonia leading to ammonia intoxication. Symptoms include vomiting, convulsions, lethargy, and poor feeding. Left untreated, patients will progress to coma and death. The age of onset and disease course depend on the severity of the defect:

- Complete enzyme deficiencies cause problems immediately after the first postnatal feed. They have high mortality.
- Less severe defects may cause symptoms only during a period of illness or with consumption of a higher-protein diet.

Treatment may include the following measures to decrease blood ammonia:

- Low protein diet.
- Levulose or lactulose, which acidify the colon, preventing absorption of ammonia.
- Antibiotics to kill ammonia-producing bacteria in the GI tract.



▲ **Figure 14–3.1A** Ornithine Transcarbamoylase Deficiency

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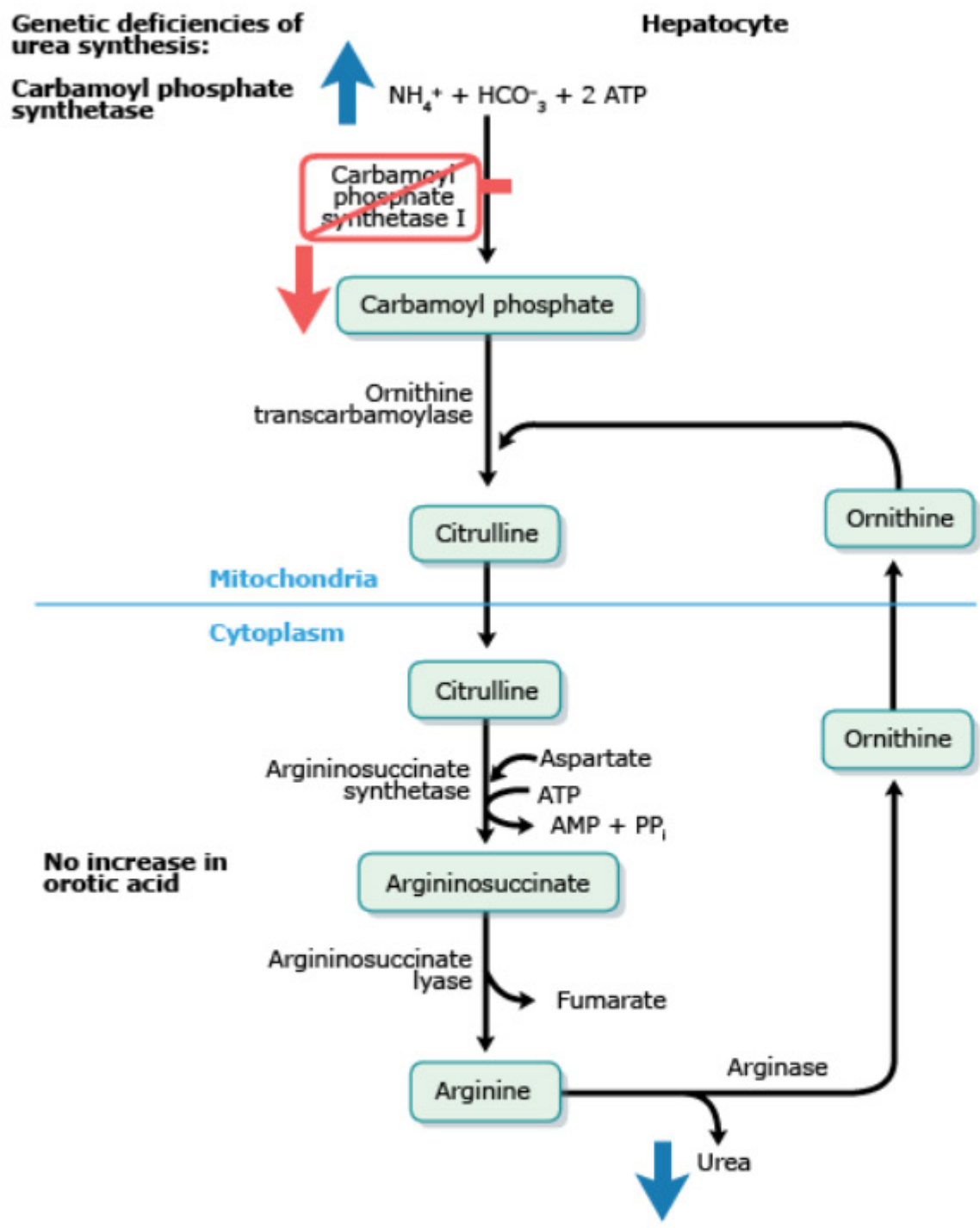
3.1.2 Carbamoyl Phosphate Deficiency

The less common deficiency of the urea cycle is carbamoyl phosphate deficiency. This condition looks the same as OTC deficiency at the clinical level except that carbamoyl phosphate deficiency will not produce orotic aciduria, and it is inherited in an autosomal recessive fashion.



▲ **Figure 14–3.1B** Carbamoyl Phosphate Synthetase Deficiency

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4 Disorders of Amino Acid Metabolism

4.1 Phenylketonuria (PKU; Phenylalanine Hydroxylase Deficiency)

PKU is an autosomal recessive disorder caused by defects in tyrosine synthesis. It is one of the most common genetic disorders in North America with a homozygote frequency of 1:11,000. The most common defect is a mutation in phenylalanine hydroxylase, with little or no residual activity. Defects in dihydrobiopterin synthesis or recycling are less common causes of PKU. These defects cause accumulation of phenylalanine, which is converted by alternate metabolic pathways to toxic metabolites, including phenylpyruvate and phenylacetate. These are particularly toxic to the nervous system, causing tremors, seizures, developmental delay, and intellectual disability. The symptoms may not be apparent until the second year of life, at which point the child already may have lost as much as 50 IQ points. For this reason, every state in the U.S. has mandatory PKU screening (for elevated plasma phenylalanine) in the neonatal period. Treatment consists of a phenylalanine-restricted diet. Patients who are diagnosed at birth and who strictly follow the diet usually have few to no symptoms. If the disease is diagnosed late, however, following the diet cannot reverse damage already done.

4.2 Alcaptonuria (Homogentisate Oxidase Deficiency)

Alkaptonuria results from a deficiency of homogentisate oxidase, the enzyme which catalyzes the breakdown of homogentisic acid to maleylacetoacetate. Normally, this would be further degraded to fumarate and acetoacetate. Deficiency of the enzyme causes the accumulation of homogentisic acid in the blood and its excretion in urine. The clinical hallmark is urine that turns dark on standing for a few hours. Patients also get arthritis from pigmented joint deposits in the cartilage (ochronosis). Treatment is symptomatic.

4.3 Maple Syrup Urine Disease (Branched-Chain Ketoacid Dehydrogenase Deficiency)

Maple syrup urine disease is an autosomal recessive disorder caused by deficiency in the 2-ketoacid dehydrogenase complex responsible for the oxidative decarboxylation of branched-chain ketoacids. This defect causes the accumulation of branched-chain amino acids and ketoacids. This accumulation is pathologic by several mechanisms:

- The ketoacids are toxic to tissues, as are the branched amino acids in excess.
- The resulting acidosis causes dysfunction of many cell processes.
- The accumulation of branched-chain amino acids inhibits transport of other amino acids, causing them to be deficient.

Symptoms occur shortly after birth and include vomiting, seizures, coma, and death. Treated patients may have varying degrees of intellectual disability. The maple syrup urine odor results from the excretion of ketoacids in the urine. Treatment consists of dietary restriction of valine, leucine, and isoleucine.

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4.4 Propionyl-CoA Carboxylase and Methylmalonyl-CoA Mutase Deficiencies

The propionic acid pathway, which is used for the metabolism of odd-carbon fatty acids, also is used for the metabolism of isoleucine, methionine, threonine, and valine. Deficiency of either propionyl-CoA carboxylase or methylmalonyl-CoA mutase, therefore, results in neonatal ketoacidosis from failure to metabolize ketoacids produced from these amino acids. The conditions are distinguished by the presence of methylmalonic aciduria in methylmalonyl-CoA mutase deficiency, and the presence of methyl citrate and hydroxypropionate in the case of propionyl-CoA carboxylase deficiency. A diet restricted in isoleucine, methionine, threonine, and valine is used in the treatment of both diseases.

4.5 Homocysteinemia/Homocystinuria

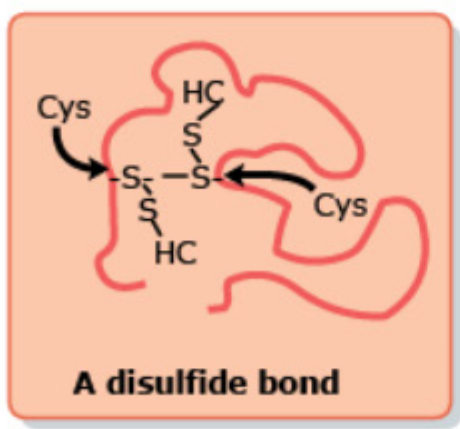
Homocystinuria is an autosomal recessive disease caused by a mutation in cystathionine β-synthase, the enzyme responsible for the catabolism of homocysteine to cystathionine. The pathologic features of the disease include:

- Ocular malformations, particularly lens dislocation.
- Musculoskeletal abnormalities, including tall stature, long fingers, pectus excavatum, and kyphoscoliosis.
- CNS defects, leading to intellectual disability and/or episodic psychosis.
- Vascular manifestations. Homocysteine is toxic to vascular endothelium, leading to recurrent thromboembolism.

All of these manifestations are due to the accumulation of homocysteine. Two molecules of homocysteine can oxidize to the disulfide cross-linked homocysteine.

First-line treatment is large doses of vitamin B6 (pyridoxine), which is a cofactor for cystathionine β-synthase. Fifty percent of patients will respond to this treatment. For the other 50%, dietary restriction of methionine is the only other treatment.

Folate deficiency, vitamin B12 deficiency, and vitamin B6 deficiency can produce a more mild form of homocysteinemia.



▲ **Figure 14–4.5A** Oxidation of Homocysteine

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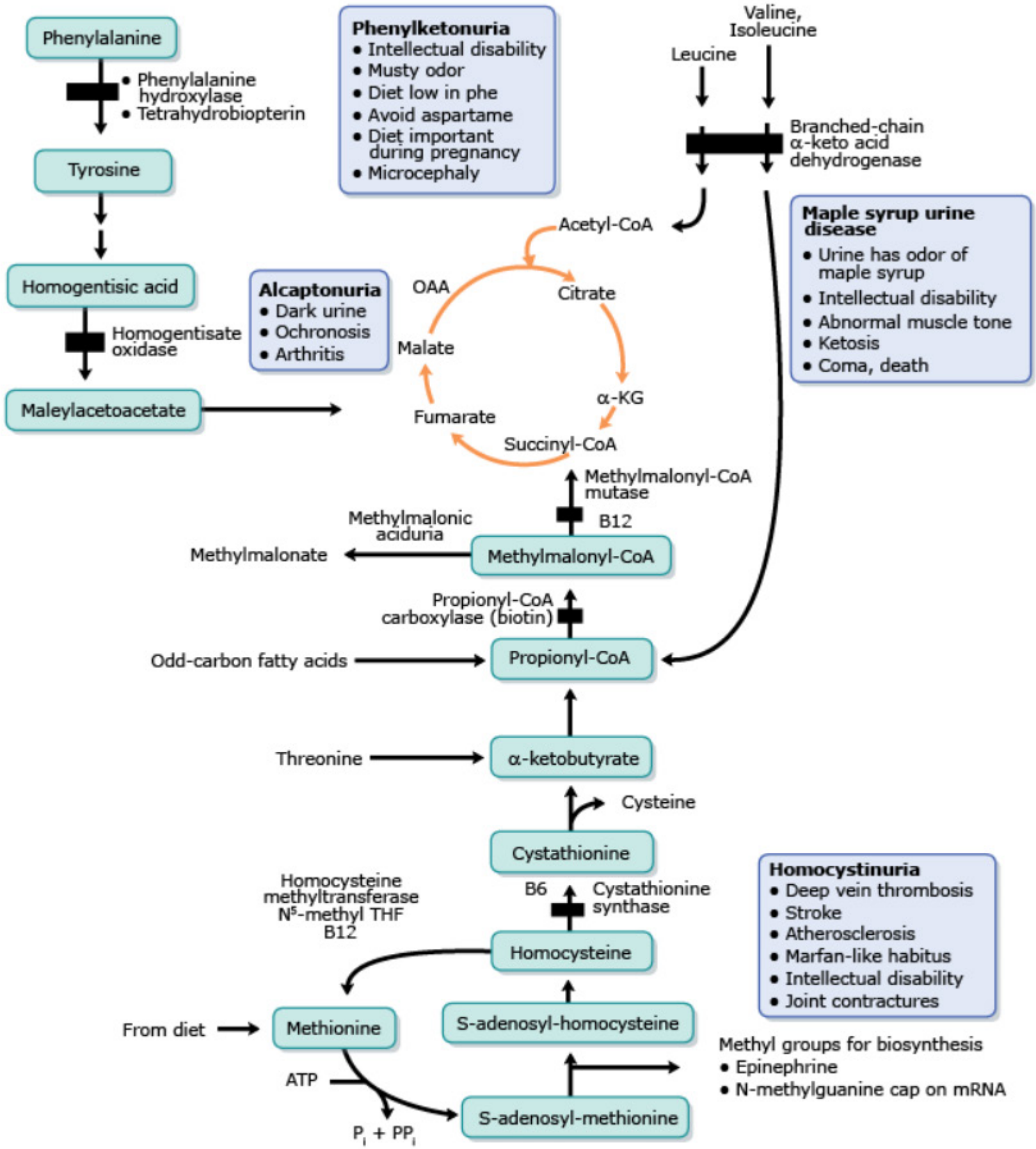

▲ **Figure 14–4.5B** Genetic Defects of Amino Acid Metabolism

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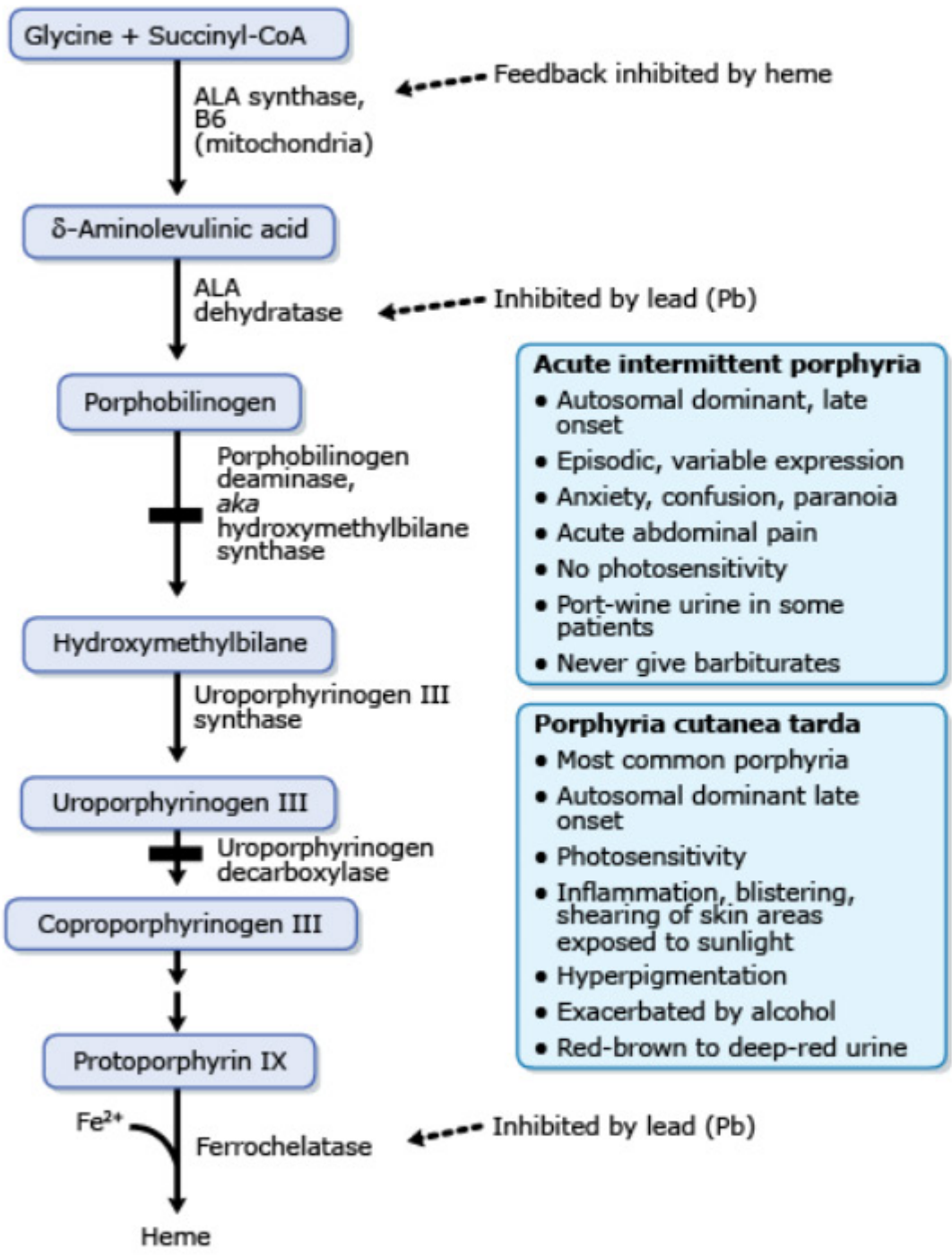
5 Heme Synthesis

The synthesis of heme proteins is essential for the production of hemoglobin, myoglobin, all the cytochromes, and the enzymes catalase and peroxidase. Heme synthesis is a complicated, multistep process that occurs in almost all the tissues of the body. In the liver, the rate-limiting enzyme δ-aminolevulinate synthase (ALA) is feedback inhibited by heme.



▲ **Figure 14–5.0** Heme Synthesis

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5.1 Defects of Heme Synthesis

5.1.1 Acute Intermittent Porphyria (AIP)

Acute intermittent porphyria is an autosomal dominant disorder caused by a defect in hydroxymethylbilane synthase (porphobilinogen deaminase) in the liver. The symptoms usually occur as intermittent attacks after puberty. They are more common in women than in men, especially during menstruation. Although the disease exhibits variable expression, symptoms include:

- Severe abdominal pain, with diarrhea or constipation and dysuria.
- Muscle weakness and paresthesias.
- Mental disturbances (insomnia, anxiety, depression, disorientation, hallucinations, and paranoia).
- Respiratory paralysis and death are rare complications.

Symptoms are thought to be caused by the toxic accumulation of ALA and porphobilinogen. These compounds are found in the urine, and turn it a red color upon standing, especially if exposed to sunlight. Acute attacks can be precipitated by infection, poor nutrition, hormonal changes, and drugs such as barbiturates, sulfonamides, and alcohol. Treatment is symptomatic with narcotics for pain and high caloric intake, which seems to increase porphobilinogen excretion. Patients should be counseled to avoid those things which tend to precipitate the attacks, and to seek prompt medical attention when attacks occur because of the threat of respiratory failure.

5.1.2 Other Porphyrias

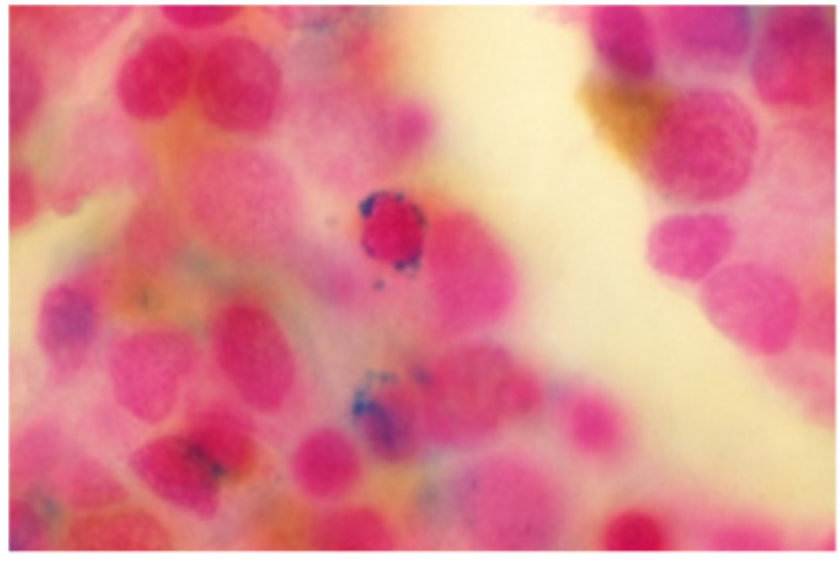
If other enzymes in the heme pathway are deficient, a variety of other porphyrias are produced for which the hallmark is photosensitive lesions. Porphyria cutanea tarda is the most common of these and is an adult-onset condition in which hepatocytes are unable to decarboxylate uroporphyrinogen. The skin lesions, which appear on sun-exposed skin, are erythematous, bullous, or urticarial. They can be severe, in some cases leading to scarring. They are a consequence of the accumulation of porphyrin intermediates in the skin.

Connection to Pharmacology

The use of barbiturates in porphyria will exacerbate the condition. This is because barbiturates are hydroxylated by the microsomal cytochrome P-450 system in the liver to facilitate their elimination from the body. With the stimulation of cytochrome P-450 synthesis, heme levels are reduced, which lessens the repression of ALA synthase and causes the production of more porphyrin precursors.

5.1.3 Vitamin B6 Deficiency

The rate-limiting enzyme of heme synthesis, ALA synthase, requires vitamin B6 for action. Aside from dietary inadequacies, other causes for this vitamin deficiency include isoniazid therapy for tuberculosis. The result will be the production of sideroblastic anemia with ringed sideroblasts.



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▶ **Figure 14–5.1A** Ringed Sideroblast

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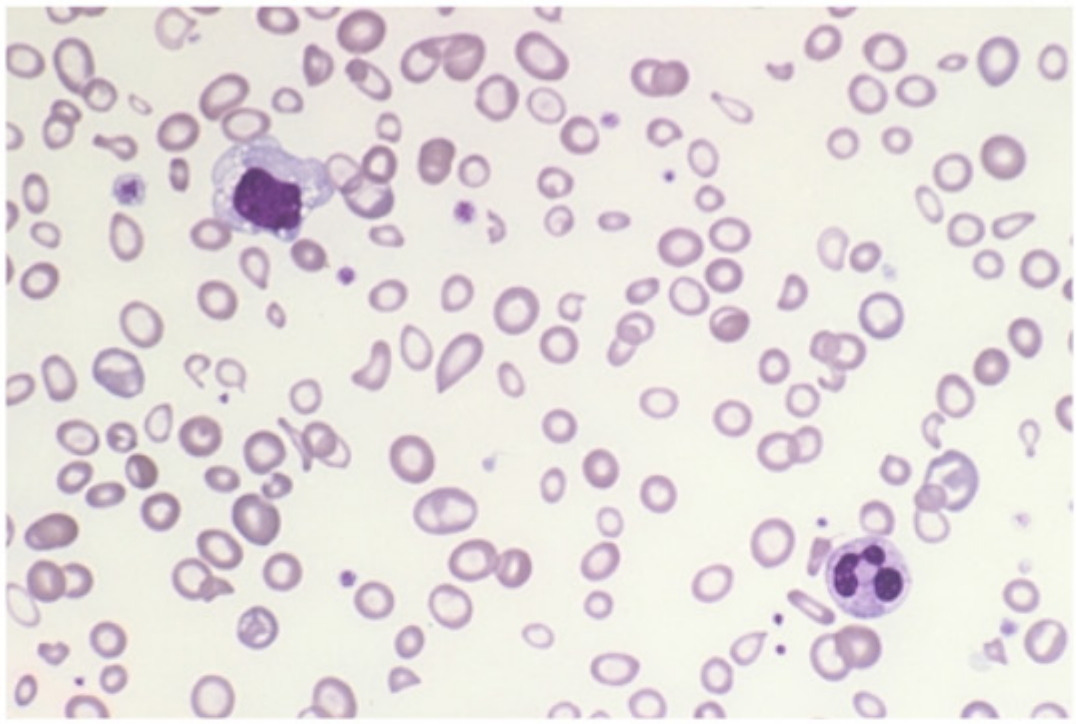
5.1.4 Iron Deficiency

Ferrochelatase, which is the last enzyme in the heme synthetic pathway, introduces Fe^{2+} into the heme ring. A deficiency of iron, therefore, will produce a microcytic hypochromic anemia.



Dr. Gladden Willis/Visuals Unlimited, Inc.

▲ **Figure 14–5.1B** Hypochromic Red Blood Cells

5.1.5 Lead Poisoning

Lead poisoning inhibits ALA dehydratase and ferrochelatase. This results in a microcytic sideroblastic anemia with ringed sideroblasts, as well as neuropathies (claw hand, wrist drop) and developmental delay. Other signs may include coarse basophilic stippling in erythrocytes, headache, nausea, memory loss, abdominal pain, diarrhea, lead lines in gums, lead deposits in the abdomen and epiphyses of long bones, and increased free erythrocyte protoporphyrin. Urine ALA levels are also increased owing to the blockage of its metabolism.

5.1.6 Comparison of Causes of Defective Heme Synthesis

▼ **Table 14–5.1** Causes of Defective Heme Synthesis

Characteristic	Iron Deficiency	Lead Poisoning	B6 Deficiency
Anemia	Microcytic	Microcytic with coarse basophilic stippling	Microcytic
Bone marrow	Disappearance of stainable iron in macrophages	Ringed sideroblasts	Ringed sideroblasts
Protoporphyrin	Elevated	Elevated	Depressed
δ-ALA	Normal	Elevated	Depressed
Ferritin	Depressed	Elevated	Elevated
Serum iron	Depressed	Elevated	Elevated
Cause	Dietary	Lead paint, pottery glaze, batteries	Isoniazid

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6 Heme Degradation

Catabolism of heme generally occurs in the liver and spleen, where old RBCs are ingested and broken down. Heme is degraded to biliverdin by the enzyme heme oxygenase, then to bilirubin by biliverdin reductase. Bilirubin is water insoluble, so hepatocytes conjugate it with glucuronic acid in order to be excreted. Conjugated bilirubin is then excreted in the bile. Intestinal bacteria convert bilirubin to urobilinogen and stercobilin. Urobilinogen is reabsorbed and excreted by the kidneys. Bilirubin and stercobilin are what gives feces the brownish color, so bile duct obstruction causes feces to be chalky white.

Accumulation of bilirubin (>2.5 mg/dL) in plasma causes *jaundice*. This is a yellow discoloration of the skin (jaundice) and sclera (icterus). Jaundice can result from the following causes.

Conjugated (direct) hyperbilirubinemia syndromes include:

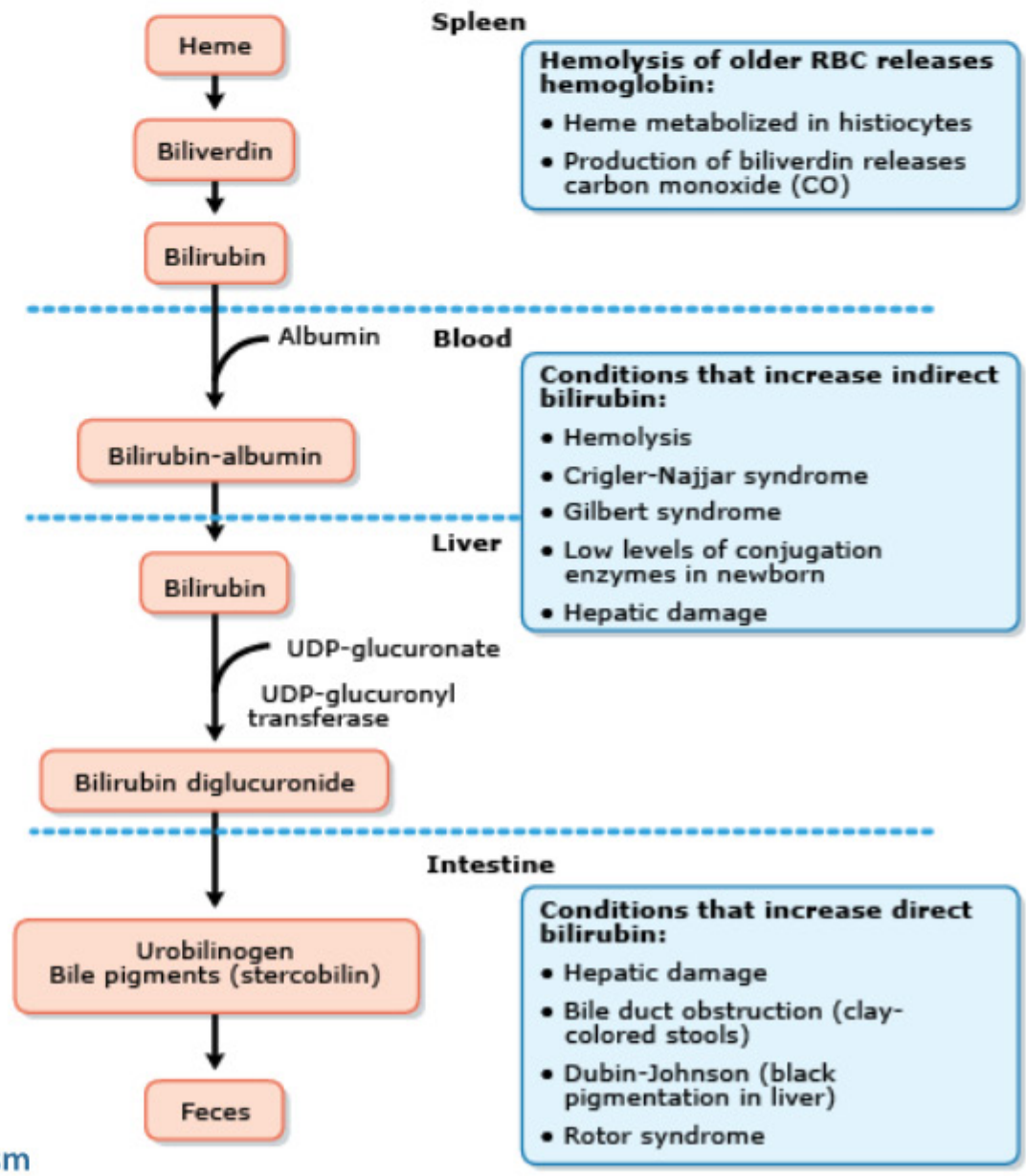
- Intra- or extrahepatic bile duct obstruction of any cause where excretion of conjugated bilirubin is blocked.
- Dubin-Johnson and Rotor syndromes are caused by defects in the transport of conjugated bilirubin into bile canaliculi.

Unconjugated (indirect) hyperbilirubinemia syndromes include:

- Hemolytic anemia: Overproduction of bilirubin overwhelms the conjugating system.
- Neonatal jaundice: Early in life, the conjugating system is not fully developed.
- Crigler-Najjar and Gilbert syndromes: Genetic defects in UDP-glucuronyl transferase cause elevation of unconjugated and total bilirubin.

Conjugated and unconjugated hyperbilirubinemia syndromes include:

- Hepatitis
- Cirrhosis



▶ **Figure 14-6.0** Heme Catabolism

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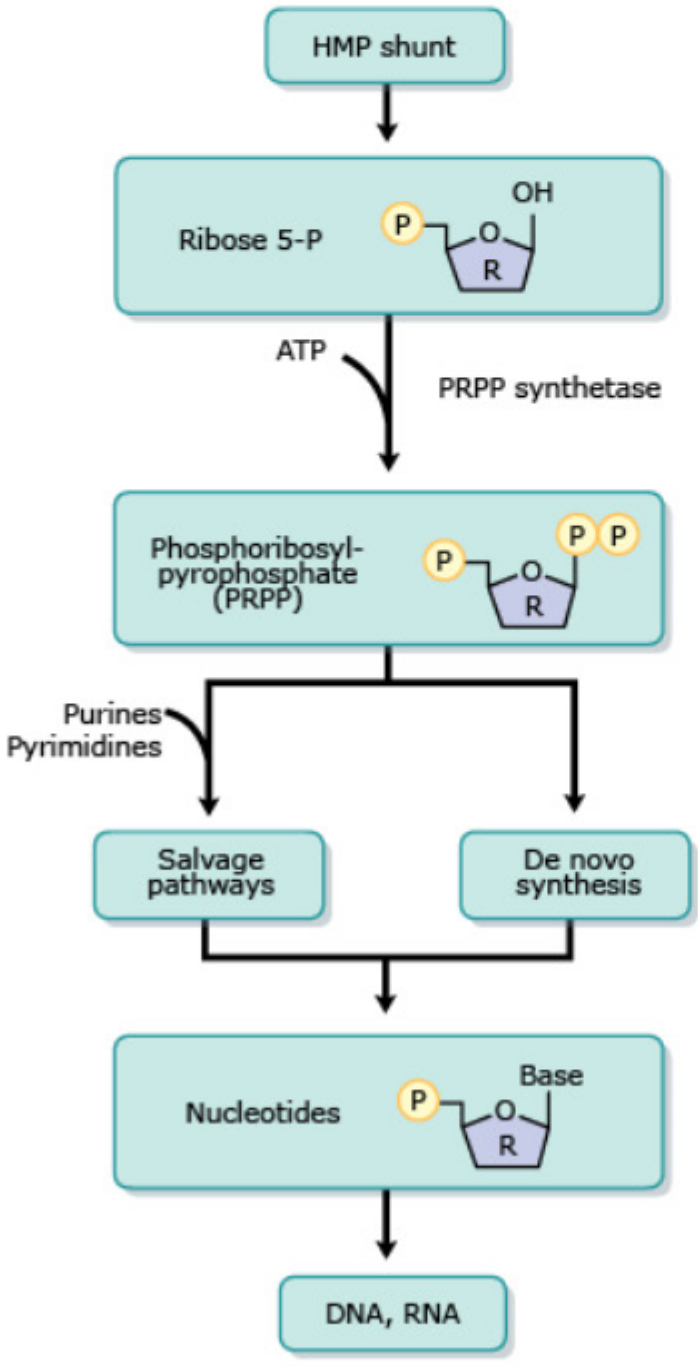
1 Overview of Nucleotide Metabolism

Nucleotides are essential for the synthesis of DNA and RNA and nucleoside triphosphates are crucial for energy transfer. Nucleotides are synthesized de novo in the liver and via salvage pathways in many cells after digestion of endogenous nucleic acids.

The hexose monophosphate shunt provides the ribose 5-phosphate necessary for nucleotide synthesis. Phosphoribosyl pyrophosphate (PRPP) is formed by addition of pyrophosphate from ATP using PRPP synthetase. In the salvage pathways, pyrimidine and purine bases are converted into nucleotides by distinct salvage enzymes.



▲ **Figure 15–1.0** Salvage and De Novo Pathways of Nucleotide Synthesis

USMLE® Key Concepts

For Step 1, you must be able to:

- Explain the de novo synthesis of pyrimidines and diagnose conditions that result from its failure.
- Describe the de novo synthesis of purines and diagnose conditions that result from its failure.
- Identify the catabolism and salvage pathways for purine and pyrimidines and the diseases that result from their failures.

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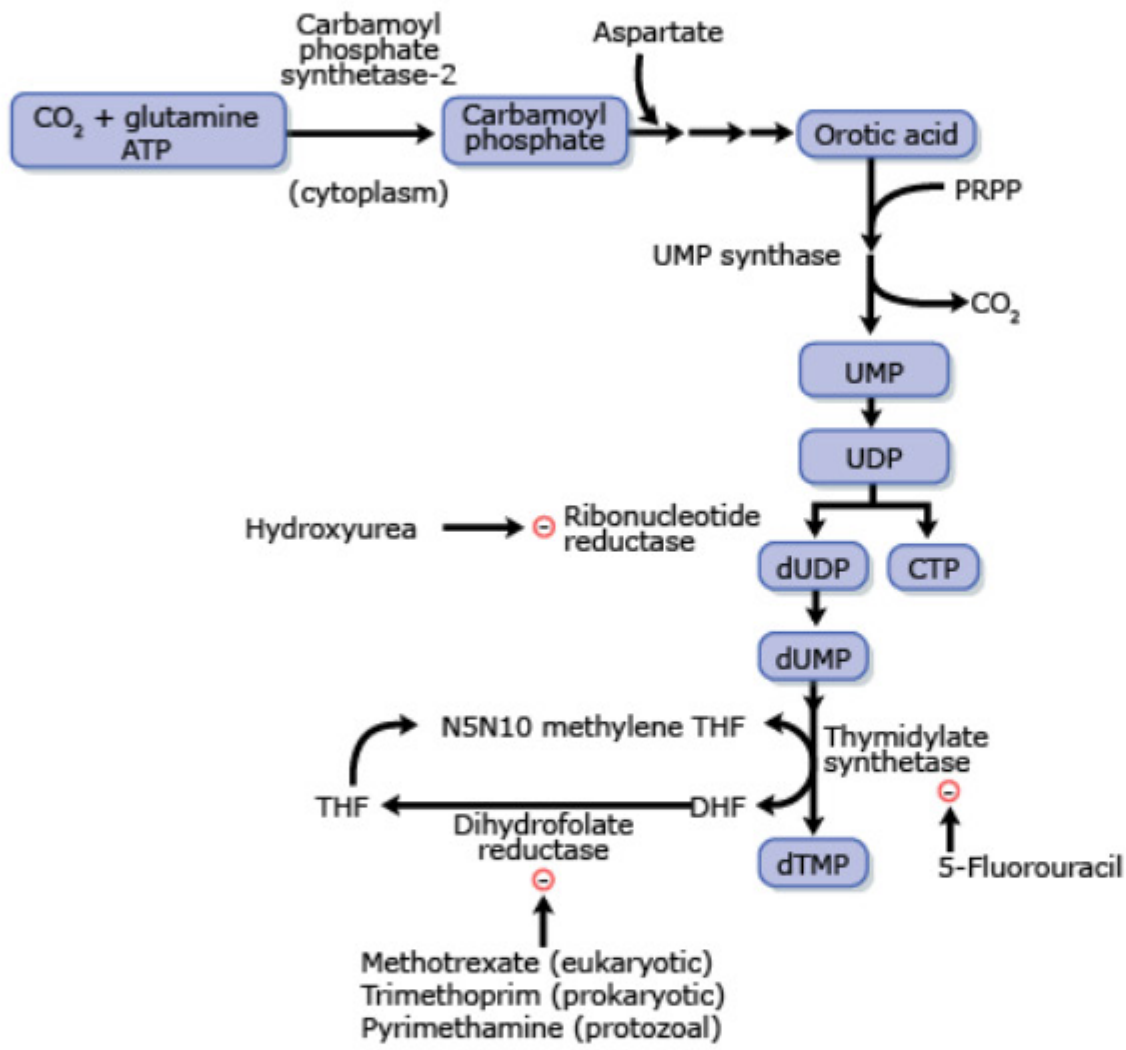
2 Pyrimidines

2.1 Synthesis

The de novo synthesis of pyrimidines occurs in the cytoplasm, beginning with aspartate, carbon dioxide, and glutamine. A carbamoyl phosphate synthetase (distinct from the mitochondrial enzyme discussed previously in the urea cycle) creates carbamoyl phosphate, to which aspartate is added to create orotic acid. The principal end product of pyrimidine synthesis is UMP.



▲ **Figure 15–2.1** De Novo Pyrimidine Synthesis

Clinical Application

Orotic Aciduria

An autosomal recessive defect in uridine monophosphate synthase causes orotic aciduria. The absence of pyrimidine synthesis impairs the formation of nucleic acids necessary for proper bone marrow hematopoiesis, so the individuals will exhibit megaloblastic anemia. The blockage of formation of UMP causes the accumulation of orotic acid, which spills over into the urine, crystallizes, and causes urinary obstruction. Treatment is by administration of uridine, which is salvaged to UMP, and feedback inhibits carbamoyl phosphate synthase-2 to prevent orotic acid accumulation. Remember that important differentials to this diagnosis are *ornithine transcarbamoylase* deficiency of the urea cycle (X-linked recessive; orotic aciduria with hyperammonemia in the absence of megaloblastic anemia) and *folate deficiency* (megaloblastic anemia in the absence of orotic aciduria).

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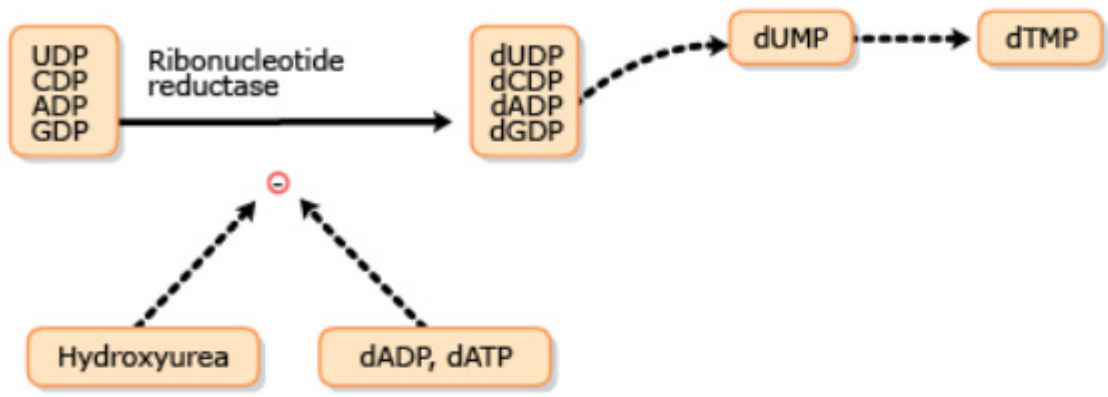
2.2 Ribonucleotide Reductase

The enzyme ribonucleotide reductase is required for the formation of all four deoxyribonucleotides for DNA synthesis. The nucleotide substrates must be diphosphates, and the enzyme is inhibited by dADP and dATP.



▲ **Figure 15–2.2** Ribonucleotide Reductase

Connection to

Pharmacology

Antineoplastic and Antimicrobial Drugs

Pathways in de novo synthesis of pyrimidines are important targets for antineoplastic as well as antimicrobial drugs:

- *Hydroxyurea* acts in S phase at the level of *ribonucleotide reductase*.
- *5-Fluorouracil* acts in S phase at the level of *thymidylate synthetase*.
- *Methotrexate* acts in eukaryotic S phase, trimethoprim acts in prokaryotes, and pyrimethamine acts in protozoa at the level of *dihydrofolate reductase*.
- When sulfamethoxazole is added to trimethoprim, the effect is a synergistic inhibition of tetrahydrofolate synthesis through two different steps:

  —Sulfamethoxazole inhibits PABA → folic acid

  —Trimethoprim inhibits DHF → THF

2.3 Pyrimidine Catabolism

Pyrimidines may be completely catabolized with the production of NH_4^+ or recycled by pyrimidine salvage pathways.

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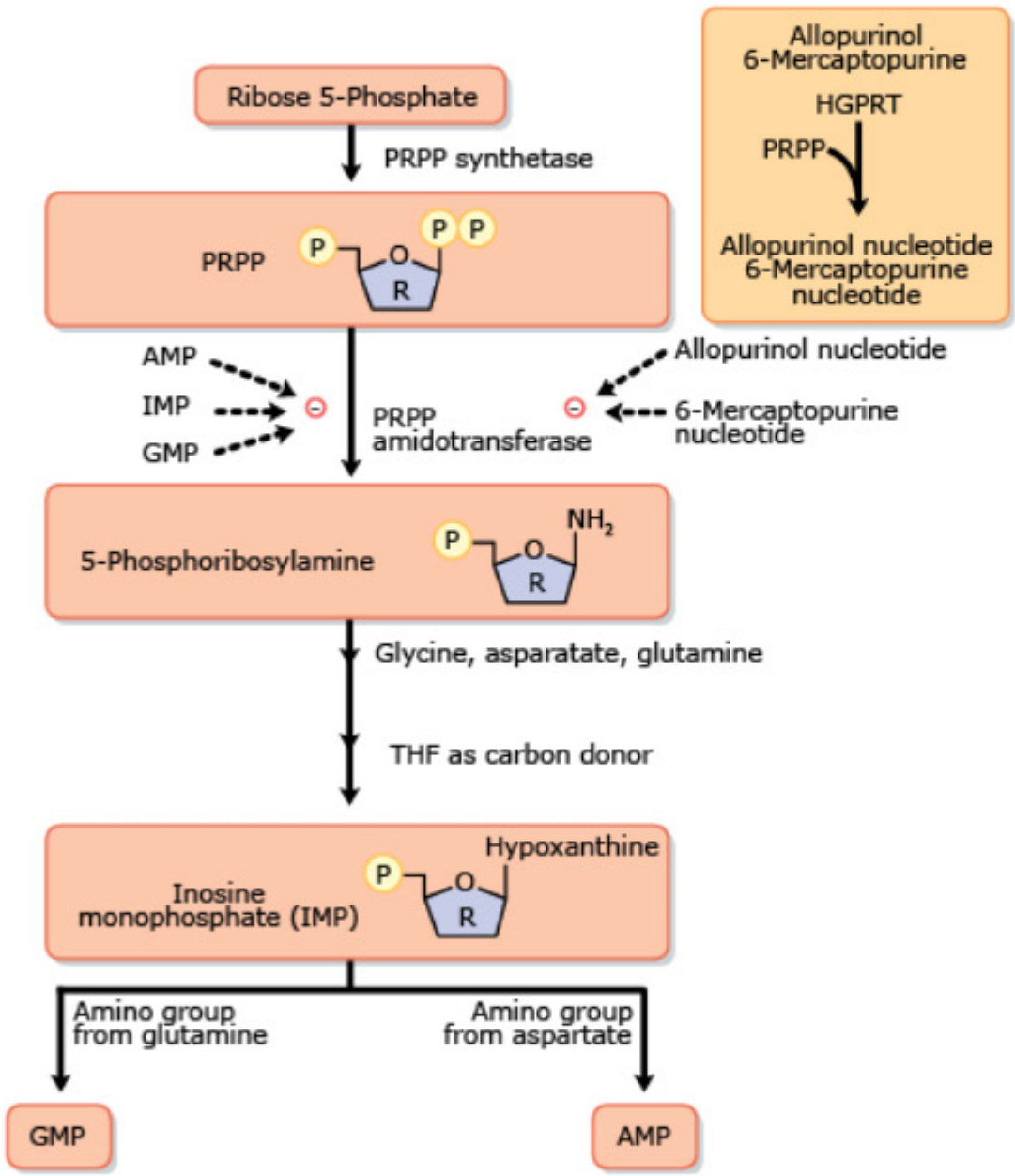
3 Purines

3.1 Synthesis

The de novo synthesis of purines begins with PRPP, and PRPP amidotransferase catalyzes the rate-limiting first step of the pathway. The amino acids aspartate, glutamine, and glycine are required for the reaction, as is tetrahydrofolate to serve as a carbon donor. Inosine monophosphate (IMP), which has hypoxanthine as its purine base, is the precursor of both GMP and AMP. Purine nucleotide end products AMP, GMP, and IMP, as well as allopurinol nucleotide and 6-mercaptopurine nucleotide, serve as inhibitors of this reaction.



▲ **Figure 15–3.1** De Novo Purine Synthesis

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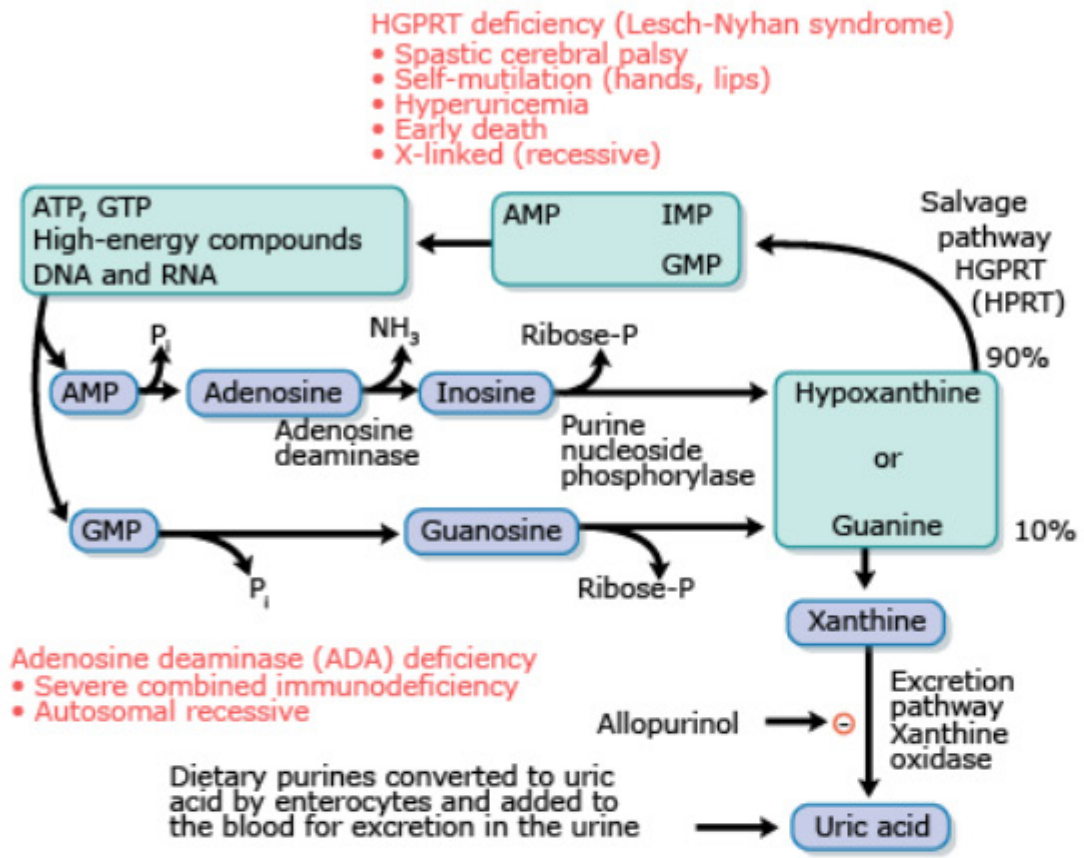
3.2 Purine Catabolism and Salvage

The release of excess purine nucleotides from DNA or RNA results in catabolism to nucleosides through the loss of P_i. Further catabolism to free bases occurs through the release of ribose or deoxyribose—90% of these purines are recycled through salvage pathways, and 10% are converted to uric acid and excreted in urine.



▲ **Figure 15–3.2** Pathways for Purine Excretion and Salvage

3.3 Diseases of Purine Catabolism and Salvage

3.3.1 Hyperuricemia and Gout

The causes of increased purine catabolism can be many, including significant cell death during chemotherapy or radiation therapy, an increase in dietary purine intake (meats and seafoods), or reduced urate absorption and unopposed urate secretion as a consequence of genetic predisposition. Any one of these causes can result in hyperuricemia, which can progress to acute or chronic gouty arthritis when monosodium urate is deposited in the joints (large toe: podagra) and adjacent soft tissues (tophi). Uric acid crystals that are needle-shaped and negatively birefringent will accumulate in these areas and stimulate neutrophilic acute inflammation. Acute attacks may thus be treated with colchicine or indomethacin to reduce the inflammation. Chronic hyperuricemia is treated with probenecid (a uricosuric drug) and allopurinol to diminish uric acid production. Allopurinol acts by inhibiting xanthine oxidase and reduces purine synthesis by inhibiting PRPP amidotransferase.

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Conditions with which hyperuricemia and gout may be associated include:

- Alcoholism (urate and lactate compete for the same transport system in the kidney)
- Galactosemia
- Glucose 6-phosphatase deficiency
- Hereditary fructose intolerance
- Lesch-Nyhan syndrome (purine salvage missing)
- Partial deficiency of HGPRT

3.3.2 Lesch-Nyhan Syndrome

Lesch-Nyhan syndrome is an X-linked recessive condition that results from any one of approximately 100 mutations within the HGPRT gene. The range of mutations includes complete deletions of the gene, point mutations that increase the K_m of hypoxanthine and guanine for the enzyme, and those which cause the enzyme to have short half-life. Without the salvaging of hypoxanthine and guanine by HGPRT, purines are passed into the excretory pathway. One of the earliest signs of the deficiency is the appearance of orange crystals of sodium urate in the diapers of an affected infant. The problem is compounded by the absence of regulatory control of the PRPP amidotranferase in the purine synthetic pathway, which results in the overproduction of purines throughout the body. The results of these failed purine salvage pathways include:

- Hyperuricemia
- Intellectual disability
- Self-mutilation (hands and lips)
- Spastic cerebral palsy
- Kidney failure and death often in the first decade

3.3.3 Adenosine Deaminase Deficiency

Adenosine deaminase (ADA) deficiency is an autosomal recessive disorder which results in severe combined immunodeficiency. dATP accumulates in cells and inhibits ribonucleotide reductase. This prevents DNA synthesis, so cells are unable to divide. Because ADA is also important in the purine salvage pathway, buildup of S-adenosylhomocysteine also contributes to lymphocyte toxicity. Because lymphocytes are some of the most actively mitotic cells in the body, they are highly susceptible to this condition. With the destruction of lymphocyte precursors, affected individuals fall prey to opportunistic pathogens and do not survive without treatment. Enzyme replacement therapy and bone marrow transplantation are the standards of care. Unfortunately, attempts at gene replacement therapy have not been lastingly successful.

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Clinical Case 1

History

A 2-year-old boy with no past medical history is brought in for a routine well-child checkup. His parents are concerned because he seems to be really tired. A review of systems shows that he seems to have some exertional dyspnea; he becomes short of breath when he is very active.

Physical Findings

- Tachycardia (increased pulse)
- Pallor (pale skin)
- Mild scleral icterus (yellow eyes)

Laboratory Results

- Normocytic anemia with spiculated red blood cells and elevated reticulocytes
- Serum methemoglobin is elevated

Diagnosis: Pyruvate kinase deficiency

Discussion

Deficiencies in glycolysis are rare. But the most common is pyruvate kinase deficiency. In this disease, there is reduced glycolytic activity. This has a particular effect in red blood cells because they have no mitochondria. Therefore, glycolysis is their only source of ATP and NADH.

ATP is crucial to the red blood cell because it powers the Na^+/K^+ ATPase, the ion transporter that maintains proper osmotic balance for the cell. In the absence of ATP, Na^+ and water are retained in the cell, causing it to swell and become rigid. Such cells are recognized as abnormal by macrophages in the spleen and liver and removed from circulation. This hemolysis is responsible for the anemia and jaundice seen in the disease.

NADH produced in glycolysis is important for maintaining iron in the proper redox state. Iron in hemoglobin is normally in the ferrous (+2) state, but in the presence of oxygen it can be oxidized to the ferric (+3) state. Hemoglobin with ferric iron is called methemoglobin and it has reduced oxygen-carrying capacity.

Most affected individuals do not require treatment, as the effects are generally mild. Severe hemolytic episodes may occur in the young or during times of physiologic stress or infection and are treated with transfusion. Chronic severe anemia can be treated by splenectomy, which decreases the hemolysis.

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Clinical Case 2

History

A 9-month-old male presents with difficulty transitioning to solid food. He has been breast-feeding without significant problems. But when he eats certain solid foods, especially fruits, he becomes very irritable and shakes, sweats, and frequently vomits.

Physical Findings

- Abdominal distension
- Jaundice

Laboratory Results

- Hypoglycemia
- Hyperuricemia
- Lactic acidosis and increased liver enzymes (AST and ALT)
- Urine is positive for reducing sugars

Diagnosis: Hereditary fructose intolerance

Discussion

There are two inborn errors of fructose metabolism. *Fructosuria* is caused by a deficiency in *fructose kinase*. This is a fairly benign condition that results in inefficient fructose metabolism. The excess fructose is excreted in the urine with no other clinical consequences.

Hereditary fructose intolerance is a more severe, even potentially life-threatening, disease. It is an autosomal recessive disorder seen in about 1 in 20,000 live births. It is caused by deficiency of *fructose 1-P aldolase (aldolase B)*. Absence of aldolase B activity leads to accumulation of fructose 1-phosphate. Among other effects, this traps a pool of phosphate so that it cannot be used to regenerate ATP, without which gluconeogenesis and glycogen synthesis are markedly suppressed, resulting in hypoglycemia. Additional metabolic disturbances cause buildup of uric and lactic acids and hepatic and renal damage.

Hereditary fructose intolerance is treated by dietary restriction of fructose and sucrose. In the absence of these sugars, fructose 1-phosphate does not build up and the clinical consequences are avoided. Controlled this way, patients live a normal life span with normal growth and intelligence. They often have an aversion to sweet foods and fruits due to the illness that these foods cause.

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Clinical Case 3

History

A 6-week-old infant is brought to her primary care physician because she is not eating well. When she does eat, she often vomits. Her mother also is concerned because the child's skin and eyes are becoming more yellow.

Physical Findings

The infant is small and ill-appearing, with obvious jaundice. She has hepatosplenomegaly and scattered bruises.

Laboratory Results

Lab tests are consistent with liver failure:

- Increased liver enzymes (AST and ALT)
- Hyperbilirubinemia
- Hypoalbuminemia
- Coagulation defects (increased PT and PTT)
- Increased galactose in urine
- Liver biopsy shows extensive fatty changes with fibrosis

Diagnosis: Galactosemia

Discussion

Galactosemia is the most common disorder of carbohydrate metabolism. It is caused by a deficiency of GALT, the enzyme responsible for catalyzing the exchange reaction between galactose 1-phosphate and UDP-glucose. The result is an accumulation of galactose 1-phosphate in the liver, where it is typically metabolized. In the absence of GALT, this excess galactose 1-phosphate is converted to toxic metabolites, particularly galactitol.

The initial clinical features appear very early, within a week of birth, because the infant's primary food source is milk. Initially, there is failure to thrive, vomiting, and diarrhea. However, as galactitol and other metabolites accumulate, there is progressive liver failure, causing hepatomegaly, jaundice, and coagulation defects. The kidneys also sustain damage, leading to progressive renal failure.

The disease is treated by giving a special diet devoid of lactose and galactose. The liver and kidney problems are limited to the first few years of life, so if the disease is controlled early, severe long-term consequences can be avoided. Consequently, all states in the United States include galactosemia in newborn screening tests. In addition to liver and kidney problems, untreated children develop cataracts from the accumulation of galactitol. They also may have intellectual disabilities, and ovarian failure in girls.

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Clinical Case 4

History

A 21-year-old man presents to an ophthalmologist complaining of worsening vision over the past few months. His optometrist was concerned about the rate of deterioration and referred him to an ophthalmologist. The ophthalmologist was even more concerned than the optometrist because the patient's mother and brother both developed blindness in early adulthood.

Physical Findings

Vision testing revealed a decrease in visual acuity in both eyes. The peripheral vision was relatively intact; the funduscopic exam showed papilledema and microangiopathic changes of the retina.

Laboratory Results

Lab tests are normal except for moderately elevated serum lactate.

Diagnosis: Leber hereditary optic neuropathy

Discussion

This disease is caused by mutations in genes encoding components of the electron transport chain. The most commonly mutated gene is the one encoding NADH dehydrogenase in complex I. These genes are encoded on the mitochondrial genome and exhibit maternal inheritance. They are passed only from mothers to children because all of the mitochondria are transmitted to the zygote via the maternal ovum. The age of onset is generally from 15 to 35 years of age.

Disruption of the electron transport chain has several effects. It compromises ATP production, which is particularly problematic in tissues that rely on aerobic metabolism for energy, such as the retina. Disruption of electron transport also increases production of reactive oxygen species that are toxic to the retinal cells.

Some patients also may exhibit a mild or moderate increase in lactate production. This is due to a buildup of NADH, which shifts pyruvate metabolism from pyruvate dehydrogenase to lactate dehydrogenase.

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Clinical Case 5

History

A 2-month-old boy is brought to his pediatrician because of increasing lethargy and decreasing appetite with weight loss. His parents also report decreasing physical activity and state that "he is kind of floppy."

Physical Findings

On exam, the child presents with tachypnea (rapid breathing). He also has poor muscle tone—he is unable to hold his head up by himself and his grasping and sucking reflexes are weak. Deep tendon reflexes are increased with clonus (involuntary repetitive muscle contractions) in the lower extremities.

Laboratory Results

Metabolic acidosis with increased anion gap.

Diagnosis: Pyruvate dehydrogenase deficiency

Discussion

Pyruvate dehydrogenase deficiency is a rare deficiency, usually of subunit E1. There are three forms of this disease:

- Neonatal onset: Overwhelming lactic acidosis resulting in death in the neonatal period.
- Infantile onset: Milder acidosis that causes neurological damage due to metabolic deficits in the brain. This occurs because of the brain's reliance on the TCA cycle for energy.
- Childhood onset: Mild form of the disease with episodic ataxia induced by high carbohydrate diet.

The form of the disease depends on the degree of residual enzyme activity. The E1 gene is on the X chromosome, but affects female heterozygotes as well as male hemizygotes. However, females tend to have less severe disease, because of random X-inactivation.

The treatment is dietary: Patients receive a high-fat, low-carbohydrate diet to minimize pyruvate generation. It also increases ketone production, an alternative energy source for the brain. Patients also receive high-dose thiamine to maximize residual enzyme activity. However, despite therapy, most neonatal- and infantile-onset patients die in the first year of life.

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Clinical Case 6

History

A 22-year-old man presents to his doctor complaining of leg cramps. He recently started a regular exercise routine, but his efforts have been limited to very short duration because of painful muscle cramps. In addition, he has noticed that his urine becomes dark for about one day after exercise and then returns to normal. He is otherwise healthy and has no other complaints.

Physical Findings

Unremarkable

Laboratory Results

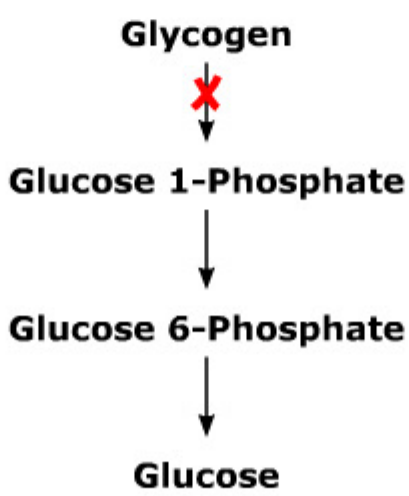
Unremarkable

Diagnosis: McArdle syndrome

Discussion

McArdle syndrome is an example of a group of disorders known as glycogen storage diseases that are caused by defects in glycogen metabolism, particularly in glycogenolysis. The clinical problems that result from these diseases are caused by the inability to mobilize glucose from liver and/or muscle and the accumulation of glycogen in these tissues, leading to cell dysfunction and death.

McArdle syndrome (type V) is the classic example of the muscular forms of glycogen storage disease. It is caused by a mutation-causing deficiency of a muscle-specific isoform of glycogen phosphorylase. Thus, during exercise, these individuals are unable to efficiently mobilize glucose through glycogenolysis and so cannot produce sufficient energy to maintain muscle activity. This leads to painful muscle cramps. Some myocytes die from insufficient energy and release myoglobin, which is cleared by the kidneys, leading to myoglobinuria, or dark urine after exercise. McArdle syndrome has a late onset, usually after age 20, and patients are otherwise healthy and have normal longevity.



▲ **Figure C–6** McArdle Syndrome

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Table A–1.1 Vitamins

Vitamin	Functions	Sources	Deficiency Syndromes	Other
Fat-Soluble Vitamins				
Vitamin A: Retinol, retinal, and retinoic acid	Retinal is an essential prosthetic group for photopigment, rhodopsin. Retinoic acid is a hormonal signal for differentiation of epithelial cells.	As beta-carotene in carrots, squash, and other yellow vegetables. Also found in fish oil, liver, broccoli, leafy greens, sweet potatoes, dairy, and eggs.	Xerophthalmia (pathologic dryness of conjunctiva and cornea), keratomalacia, and nyctalopia (night blindness).	Vitamin A deficiency is the most common cause of blindness in children worldwide.
Vitamin D3: Cholecalciferol	Precursor of calcitriol, a sterol hormone that regulates calcium and phosphorous in response to changes in parathyroid hormone.	Skin exposure to ultraviolet light converts 7-dehydrocholesterol to cholecalciferol. Also can be obtained from diet (fish oils and fortified dairy products). Must be converted to calcitriol by sequential metabolism in liver and kidney.	Rickets (soft bones) in infants and osteomalacia (brittle bones) in adults.	Vitamin D2, produced from ergosterol in fungi, is a less potent form of the vitamin.
Vitamin E: α-tocopherol	Incorporated into VLDL by liver; believed to act as antioxidant, particularly of membrane- or lipoprotein-associated lipids.	Almonds, hazelnuts, avocados, carrots, spinach, and plant oils such as olive and canola.	Red blood cells more susceptible to osmotic lysis. Neuromuscular problems.	Relatively rare deficiency disorder due to mutation in gene for TTP (tocopheryl-transfer protein) manifests in neurological disturbance.
Vitamin K: Phylloquinone (K1) or menaquinone (K2)	Cofactor for γ-glutamyl-carboxylation (GLA) of clotting factors II, VII, IX, X, protein C, and protein S.	Broccoli, kale, and spinach. Also produced by intestinal flora.	Bleeding diathesis with prolonged prothrombin time (PT).	Deficiency common in newborns (pre-intestinal colonization) and with broad-spectrum antibiotic use due to lack of adequate intestinal flora.

(continued)

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Table A–1.1 Vitamins *(continued)*

Vitamin	Functions	Sources	Deficiency Syndromes	Other
Water-Soluble Vitamins				
Vitamin B1: Thiamine	Cofactor for dehydrogenases; e.g., pyruvate dehydrogenase, α-ketoglutarate dehydrogenase, branched-chain amino acid dehydrogenase, and transketolases.	Yeast, yeast extract, lean pork, oats, flax, rye, fortified cereals, lentils, beans, potatoes, asparagus, cauliflower, oranges, liver, eggs.	• Wet beriberi: Cardiomyopathy and vasodilation progressing to congestive heart failure. • Dry beriberi: Peripheral sensory neuropathy, weakness, and hyporeflexia. • Wernicke-Korsakoff syndrome (cerebral beriberi): Confusion, ataxia, and nystagmus.	Common in malnutrition where polished rice is the staple grain and in chronic alcoholics. Remember to give thiamine before glucose when treating alcoholic hypoglycemia.
Vitamin B2: Riboflavin	Cofactor, as FAD (flavin adenine dinucleotide) and FMN (flavin mononucleotide), and electron carrier for redox reactions (succinate dehydrogenase, electron transport, citric acid cycle, and β-oxidation of fatty acids).	Dairy, green leafy vegetables, liver, malted barley, legumes, mushrooms, almonds, and eggs. Richest natural source is yeast.	Dermatitis, angular cheilosis (drying and cracking of the angles of the mouth), and glossitis (enlargement and inflammation of the tongue).	
Vitamin B3: Niacin, nicotinic acid, nicotinamide	Cofactor, as NAD^+ (nicotinamide adenine dinucleotide) and $NADP^+$ (nicotinamide adenine dinucleotide phosphate), for redox reactions (isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase).	Dairy, meat, nuts, and eggs. Tryptophan can be converted to niacin in the body, but inefficiently.	Pellagra: Scaly dermatitis, diarrhea, dementia, and death (the four Ds).	Used clinically as a hypolipidemic agent at high doses.
Vitamin B5: Pantothenate	Cofactor as coenzyme A in acyl group transfer, and as fatty acyl transferase in fatty acid synthase.	Meat, fish, broccoli, egg yolks, and yeast.	Deficiency is practically unknown, except in extreme general malnutrition. Experimental human deprivation results in fatigue, listlessness, and peripheral neuropathy.	

(continued)

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Table A–1.1 Vitamins *(continued)*

Vitamin	Functions	Sources	Deficiency Syndromes	Other
Water-Soluble Vitamins				
Vitamin B6: Pyridoxine, pyridoxal phosphate	As pyridoxal phosphate, serves as a coenzyme for synthesis of amino acids, neurotransmitters (serotonin, norepinephrine), sphingolipids, and aminolevulinic acid (heme synthesis).	Salmon, chicken, potatoes, bananas, and fortified foods.	Deficiency causes sideroblastic anemia, dermatitis, peripheral neuropathy, and convulsions.	Isoniazid (INH), an anti-mycobacterial agent, interferes with pyridoxal phosphate metabolism, and can cause deficiency.
Vitamin B9: Folate	Cofactor for one-carbon metabolism and synthesis of thymidine (DNA).	Green leafy vegetables, legumes, and fortified cereals.	Megaloblastic anemia. Deficiency in pregnancy increases risk of neural tube defects.	Red blood cell folate is superior to serum folate as a measure of deficiency.
Vitamin B12: Cobalamin	Required for only two human enzymes: Methylmalonyl-CoA mutase and methionine synthase.	Liver and fortified nutritional yeast.	Megaloblastic anemia and neuropathy due to posterior column demyelination (causing ataxia and paresthesias).	Deficiency commonly caused by autoimmune destruction of parietal cells that produce intrinsic factor required for B12 absorption (pernicious anemia).
Vitamin C: Ascorbate, L-ascorbic acid	Cofactor for lysyl and prolyl hydroxylase enzymes, which are essential for collagen synthesis.	Citrus, green and red peppers, broccoli, and tomatoes.	Scurvy: Easy bruising, bleeding gums, poor wound healing, and lethargy.	

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Genetics

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1 The Language of Mendelian Inheritance

1.1 Chromosome

A thread-like linear strand of DNA bonded to various proteins in the cell nucleus that contains the genetic message passed down from generation to generation. Humans have 23 pairs of chromosomes, with one of each pair *(homologous chromosomes)* contributed by each parent, yielding a total of 46 chromosomes per cell. One of the 23 pairs of chromosomes is made up of two sex chromosomes, X and Y. A female has two X chromosomes, while a male has one X and one Y chromosome. The remaining 22 pairs of chromosomes are termed *autosomes* and are present in both males and females.

1.2 Gene

Genes are the basic units of heredity. On a molecular level, genes are made up of specific segments of DNA that encode a specific protein or non-translated RNA (rRNA, tRNA, and snRNA).

1.3 Allele

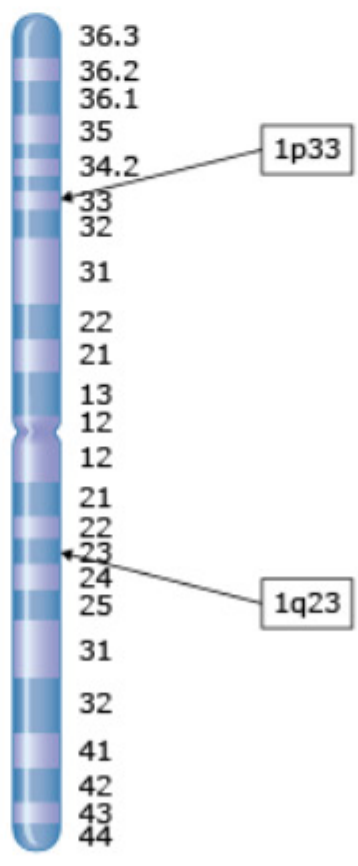
An allele is an alternative form of a single gene usually caused by a difference of one or a few nucleotides. If an individual has the same allele on both homologous chromosomes, they are said to be *homozygous* for that allele. If the individual has different alleles, they are said to be *heterozygous.* When there are multiple alleles of a single gene within a population, the allele is said to be *polymorphic.*

1.4 Locus

A locus is the specific location of a gene on a chromosome. Specialized staining techniques reveal characteristic banding patterns for each chromosome. The bands are then numbered allowing us to define specific physical locations, or addresses, on individual chromosomes.

1.5 Genotype

The *genotype* of an individual is a description of the alleles carried at a particular locus.



▶ **Figure 1–1.4** Gene Loci

USMLE® Key Concepts

For Step 1, you must be able to:

- Explain the meaning of chromosome, gene, allele, locus, genotype, phenotype and mutation.
- Identify the five modes of inheritance of single-gene disorders, namely autosomal dominant and recessive, X-linked dominant and recessive, and mitochondrial.
- Evaluate recurrence risk for each of the five modes of inheritance of single-gene disorders.

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1.6 Phenotype

The *phenotype* of an individual refers to the physical or functional manifestation of the genotype. A *dominant* allele is one that expresses its phenotype in either the homozygous or heterozygous state. A *recessive* allele is one that expresses its phenotype only in the homozygous state. *Codominant* alleles are those express both of their phenotypes together in the heterozygous state.

Clinical Application

Dominant, Recessive, and Codominant Alleles

In the determination of eye color, the brown allele is dominant to the blue allele. Thus, both homozygous brown and heterozygous brown/blue will express a brown phenotype. Only the homozygous blue genotype will produce a blue phenotype. In the case of the ABO blood glycoproteins, A and B alleles are expressed codominantly, so the individual with heterozygous genotype will express an AB phenotype. The O allele is recessive, so only homozygous O individuals will express the O phenotype. Major histocompatibility alleles (the most polymorphic gene system in the human species) are also expressed codominantly.

1.7 Mutation

A *mutation* is a change in the DNA sequence. If mutations happen during gametogenesis, they can be transferred vertically to the next generation in the form of new alleles. A *missense* mutation will result in the substitution of an amino acid in a polypeptide chain, whereas a *nonsense* mutation will produce a stop codon, and thus cause the production of a truncated protein product. If bases are added or deleted in multiples of three, the mutation is said to be *in-frame,* if not, the mutation will result in *frame shift.* If a mutation results in the production of a protein with additional or new function, the mutation is said to be a *gain-of-function* mutation. If a mutation results in the loss of production or diminished activity of a protein, it is said to be a *loss-of-function* mutation.

1.8 Recurrence Risk

Recurrence risk is the probability that the offspring of a couple will express a genetic disease. Since each offspring produced is an independent event, the recurrence risk remains the same regardless of the previous proportion of affected versus unaffected children. Recurrence risks will be different for conditions that are inherited as dominant versus recessive and autosomal versus sex-chromosome-linked traits.

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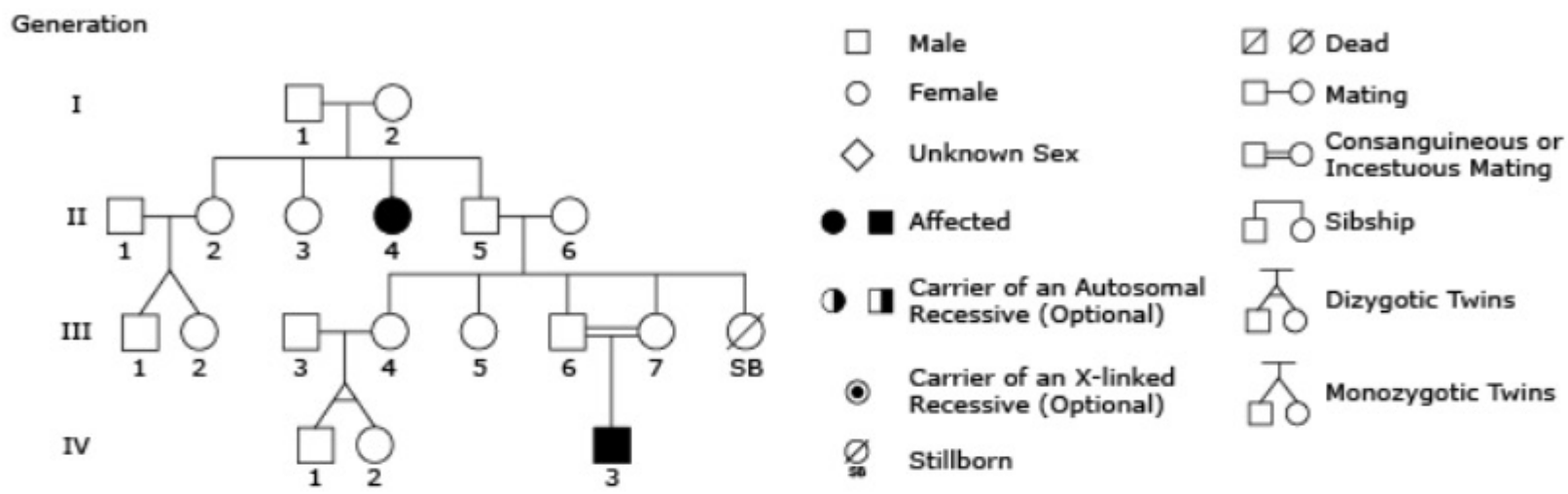
2 Basics of Pedigree Analysis

A pedigree is a diagram of relationships, or family tree, with which the transmission of a particular phenotype can be evaluated. Observation of the passage of particular phenotypes through generations can provide inferences about the genotypes present—for example, whether transmission occurs in a dominant or recessive fashion.

The convention for pedigree iconography is shown in figure 1–2.0.



▲ **Figure 1–2.0** Pedigree Iconography

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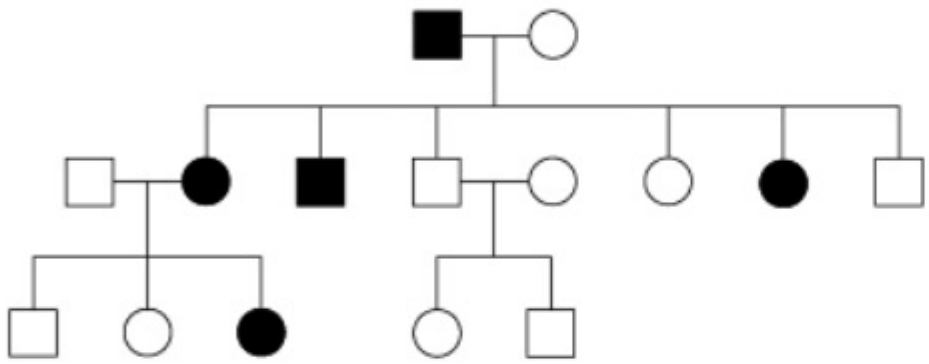
3 Modes of Inheritance

3.1 Autosomal Dominant Inheritance

3.1.1 Identification of the Pedigree

Autosomal dominant disorders are inherited in a dominant fashion—any individual that receives even one disease causing allele will be affected. Therefore, affected patients can be either homozygous or heterozygous for the disease causing allele. There is no carrier state and unaffected individuals are by definition homozygous for the "normal" allele.

- In order to inherit the disorder, at least one parent must be affected.
- The disease is generally observed in multiple generations.
- Males and females are affected in roughly equivalent proportions.



▲ **Figure 1–3.1A** Pedigree of an Autosomal Dominant Inheritance

3.1.2 Calculating the Recurrence Risk

To calculate the recurrence risk for an autosomal dominant disease, a Punnett square is constructed using the potential gamete contributions of each parent. By convention, the dominant allele is represented by a capital letter and the recessive allele by a lower case letter. In this case, the most common mating would be one between an affected heterozygote and a normal homozygote, and the recurrence risk would be 50%. If both parents were heterozygous, the recurrence risk would be 75%.

	a	a
A	Aa	Aa
a	aa	aa

▲ **Figure 1–3.1 B** Recurrence Risk for an Autosomal Dominant Mating

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Clinical Application

Diseases with Autosomal Dominant Inheritance

There are three potential mechanisms by which autosomal dominant diseases cause pathology. Those which act as *gain-of-function* mutations cause the production of a protein with adverse function (e.g., Huntington disease). In cases where there is a *dominant negative*, the mutated gene product blocks the function of the normal gene product (e.g., osteogenesis imperfecta). In some cases *haploinsufficiency* is the cause, since the one normal allele product is not sufficient to maintain normal function (e.g., familial hypercholesterolemia). Examples of important autosomal dominant diseases include:

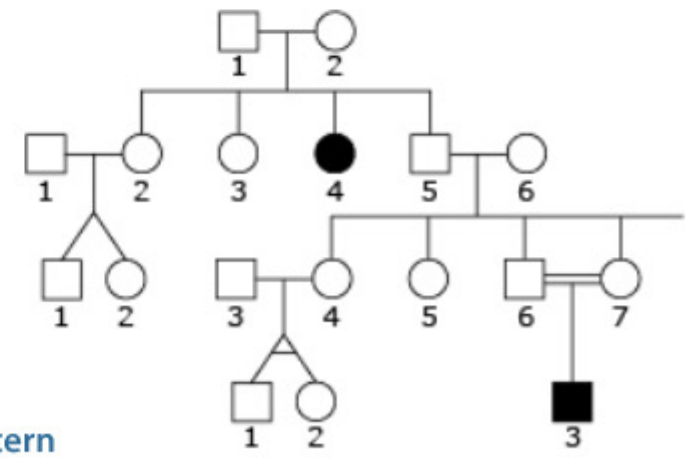
- Acute intermittent porphyria
- Familial hypercholesterolemia
- Huntington disease
- Marfan syndrome
- Neurofibromatosis type 1

3.2 Autosomal Recessive Inheritance

3.2.1 Identification of the Pedigree

In autosomal recessive disorders, the disease causing allele exhibits a recessive genotype-phenotype relationship. This means that in order to be afflicted with the disease, an individual must be homozygous for the disease-causing allele. In heterozygous individuals with a dominant allele present, the disease does not occur. These heterozygous individuals are termed *carriers* because, despite not having the disease, they carry the abnormal allele in their genome and can pass it on to future generations.

- Offspring must inherit one copy of the disease-causing allele from each parent.
- The disease phenotype often skips generations.
- Males and females will be affected in roughly equal proportions.
- It is more common in consanguineous (incestuous) matings.



▶ **Figure 1–3.2A** Autosomal Recessive Inheritance Pattern

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3.2.2 Calculating Recurrence Risk

Because mutated alleles are usually rare in the population, the most common scenario for the disease is in the offspring of two carrier (heterozygous) parents. In such a pairing, the probability of affected offspring is 1 in 4, or 25%. The probability of carrier offspring is 2 in 4, or 50%:

	A	a
A	AA	Aa
a	Aa	aa

▲ **Figure 1–3.2B** Recurrence Risk for an Autosomal Recessive Mating

If only one parent is a carrier, none of the children will have the disease, but half will be carriers.

	A	a
A	AA	Aa
A	AA	Aa

If one parent is affected and the other is homozygous normal, no children will have the disease, but all will be carriers.

	a	a
A	Aa	Aa
A	Aa	Aa

If one parent is affected and the other is a carrier, half of the children will have the disease and half will be carriers.

	a	a
A	Aa	Aa
a	aa	aa

▲ **Figure 1–3.2C** Examples of Recurrence Risk for Autosomal Recessive Mating

3.2.3 Recurrence Risk for Known Phenotype

If an individual in an affected pedigree is known to be phenotypically normal, then his genotype must be Aa, aA, or AA. We know he cannot be aa, because he is phenotypically normal. Therefore, his risk of being a carrier is 2/3, or 67%.

Clinical Application

Diseases with Autosomal Recessive Inheritance

Most recessive diseases are due to loss-of-function mutations in genes encoding enzymes. Typically, these pathways can function normally even if half of the normal amount of enzyme is being produced. Important examples:

- Cystic fibrosis
- Phenylketonuria
- Sickle cell anemia
- Tay-Sachs disease

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3.3 X-Linked Recessive Inheritance

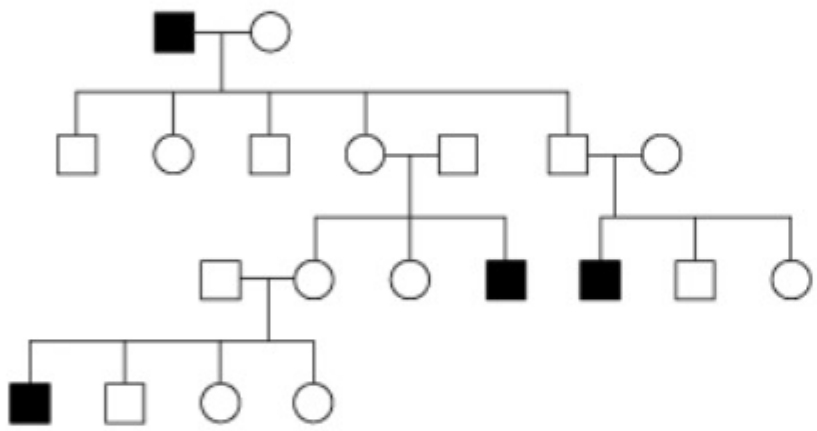
3.3.1 Identification of the Pedigree

Much like autosomal recessive disorders, X-linked recessive disorders are typically the result of loss-of-function mutations. However, the location of the disease-causing gene on the X chromosome means that there will be different inheritance patterns for males and females.

In females, who have two X chromosomes, X-linked recessive inheritance occurs similarly to autosomal recessive inheritance. Specifically, two copies of the disease-causing allele are necessary in order to have the disease. In other words, homozygous females are affected, but heterozygous females are carriers.

Males, on the other hand, are *hemizygous* for all of the alleles on the X chromosome. Thus if a male inherits the disease-causing allele, there is no normal allele to compensate and the individual is affected with the disease.

- X-linked recessive diseases are seen much more commonly in males than in females.
- Skipped generations are common.
- Male-to-male transmission is not observed.



▲ **Figure 1–3.3A** X-Linked Recessive Inheritance Pattern

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3.3.2 Calculating Recurrence Risk

In the cross between a carrier mother and an unaffected father, 50% of the sons will be affected and 50% of the daughters will be carriers. Disease allele is lowercase a.

	X^A	Y
X^A	X^AX^A	X^AY
X^a	X^AX^a	X^aY

In the cross between a normal mother and an affected father, 100% of the daughters will be carriers.

	X^a	Y
X^A	X^AX^a	X^AY
X^A	X^AX^a	X^AY

In the cross between a carrier mother and an affected father, half of the daughters will be carriers and half will be affected. Half of the sons will be affected.

	X^a	Y
X^A	X^AX^a	X^AY
X^a	X^aX^a	X^aY

▲ **Figure 1–3.3B** Examples of Recurrence Risk for X-Linked Recessive Disorders

Clinical Application

Diseases With X-Linked Recessive Inheritance

These diseases generally result from a loss-of-function mutation in genes located on the X chromosome. Since males are hemizygous for the X chromosome, if they receive a mutated X chromosome, they will express disease. Examples of important X-linked recessive diseases include:

- Bruton agammaglobulinemia
- Duchenne muscular dystrophy
- G6PD deficiency
- Hemophilia A and B
- Lesch-Nyhan syndrome
- Menkes disease
- Ornithine transcarbamoylase deficiency
- IL receptor γ chain SCID

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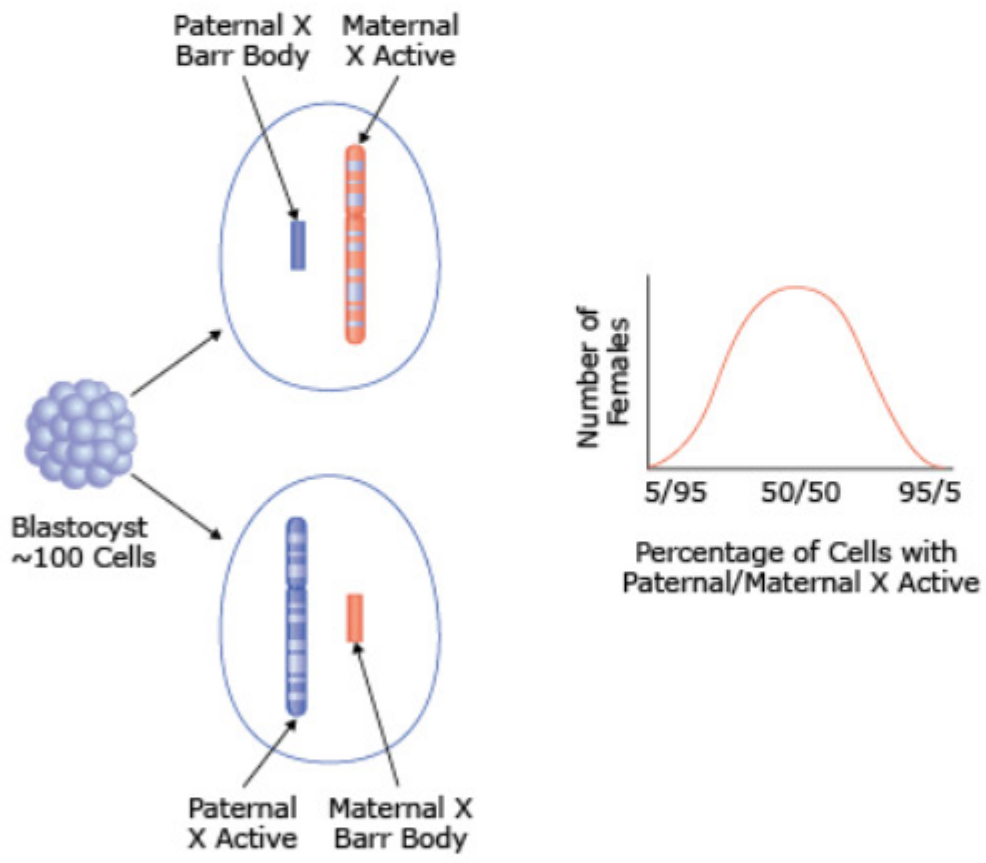
3.3.3 X Inactivation

In contrast to autosomes, in females one X chromosome of a pair undergoes a process called X inactivation. X chromosome inactivation occurs at the blastocyst stage of female embryo development, and results in a highly condensed structure known as a Barr body. Inactivation occurs in each cell of the blastocyst in a pattern that is:

- *Random:* In the blastocyst stage, cells have no preference and may inactivate either the paternal or maternal X chromosome.
- *Fixed:* After the initial inactivation of an X chromosome in a cell at the blastocyst stage, all future cells derived from that cell will maintain the same pattern of X chromosome inactivation.
- *Incomplete:* Although the inactivated X chromosome is condensed into a Barr body, some sections are still transcribed.
- *All X chromosomes are inactivated except one.* In cases where a female has three X chromosomes, there will be two Barr bodies in each cell.
- The *XIST gene* has been identified as the primary cause of X inactivation. This gene produces an RNA product that coats the chromosome, encourages its condensation into heterochromatin and methylation of specific gene regions.



▲ **Figure 1–3.3C** X Inactivation

3.3.4 Manifesting Female Heterozygotes

Based on the phenomenon of X chromosome inactivation, it is possible for a minority of females to be affected by an X-linked recessive disease. These females are referred to as *manifesting heterozygotes.* If random inactivation results in silencing of the normal allele, then the female becomes effectively hemizygous at that allele and develops the disease. The severity of the disease, however, is often less than that seen in males because inactivation of the normal allele does not occur in every cell.

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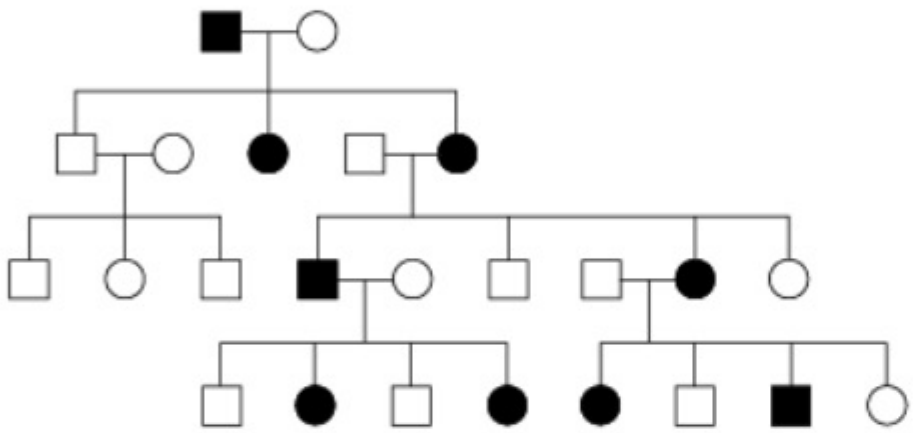
3.4 X-Linked Dominant Inheritance

3.4.1 Identification of the Pedigree

X-linked dominant disorders result in similar pathogenicity as those involved in autosomal dominant disorders: gain-of-function, dominant negative, and haploinsufficiency. However, due to the location of the disease-causing gene on the X chromosome, the patterns of inheritance will be distinct.

- Females heterozygous and homozygous for the disease-causing allele will be affected.
- Disease is seen about twice as often in females as in males.
- Males are hemizygous for the X chromosome, so all those with the affected allele will have the disease.
- As with autosomal dominant disorders, there is no carrier state.
- Skipped generations are uncommon.



▲ **Figure 1–3.4A** X-Linked Dominant Inheritance Pattern

3.4.2 Calculating Recurrence Risk

Affected fathers pass the disease to all of their daughters, but not to their sons. Disease allele is lowercase a.

	X^A	Y
X^a	X^AX^a	X^aY
X^a	X^AX^a	X^aY

Affected mothers can pass the disease to half of both daughters and sons.

	X^a	Y
X^A	X^AX^a	X^AY
X^a	X^aX^a	X^aY

▲ **Figure 1–3.4B** Examples of the Recurrence Risk for X-Linked Dominant Disorders

Diseases With X-Linked Dominant Inheritance

There are relatively few diseases with X-linked dominant inheritance. These are caused by gain-of-function, dominant negative, or haploinsufficiency mutations on the X chromosome. Important examples include:

- Fragile X syndrome
- Hypophosphatemic rickets
- Pyruvate dehydrogenase deficiency

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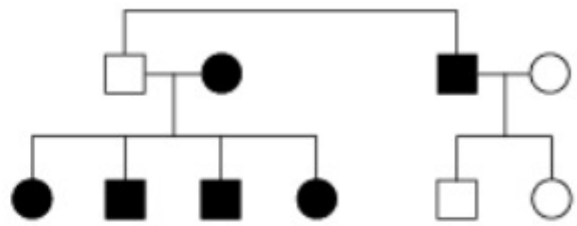
3.5 Mitochondrial Inheritance

3.5.1 Identification of the Pedigree

Although most of a cell's DNA is contained within the nucleus, mitochondria each contain their own circular chromosome. This mitochondrial DNA encodes proteins important in the electron transport and oxidative phosphorylation systems as well as several tRNA and rRNA molecules.

During fertilization, the sperm and oocyte each contribute one set of 23 chromosomes. However, the sperm does not contribute any mitochondria. Thus, all of the mitochondria—and therefore all the mitochondrial DNA—come from the oocyte. Inheritance occurs only through mothers and is therefore matrilineal. As a result:

- All offspring of an affected female will be affected.
- Both males and females are affected.
- None of the offspring of an affected male are affected.



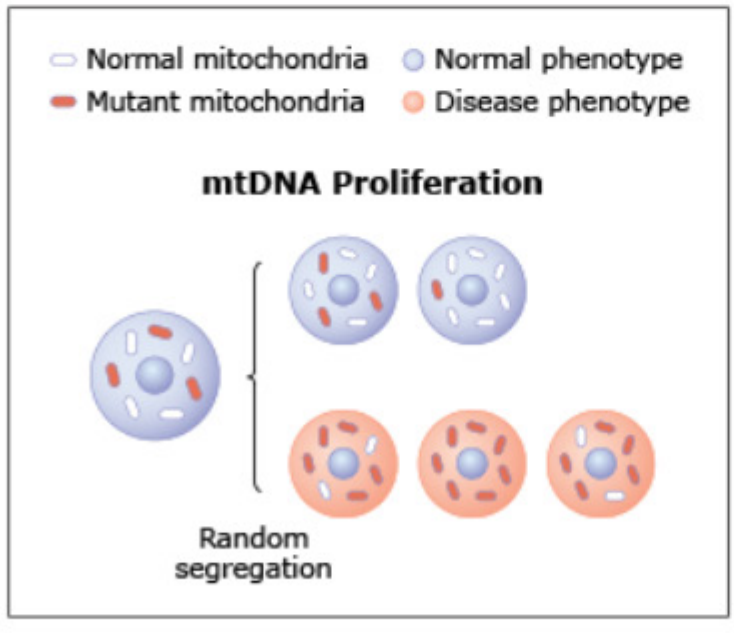
▲ **Figure 1–3.5A** Mitochondrial Inheritance Pattern

3.5.2 Calculation of the Recurrence Risk

All of the children of an affected mother inherit the disease, whereas none of the children of an affected father develop the disease.

3.5.3 Heteroplasmy

Since each mitochondrion carries its own copy of the mitochondrial genome, and since there are many thousands of mitochondria in each cell, mutations can arise in some mitochondria and not in others. During cell division individual mitochondria are segregated into daughter cells, resulting in some cells with a majority of "normal" mitochondria and some cells with a majority of mitochondria harboring the disease-causing mutation. This phenomenon is known as *heteroplasmy* and can lead to variations in the expression of mutated mitochondrial genes among cells, and therefore variation in the severity of disease among individuals.



▲ **Figure 1–3.5B** mtDNA Proliferation

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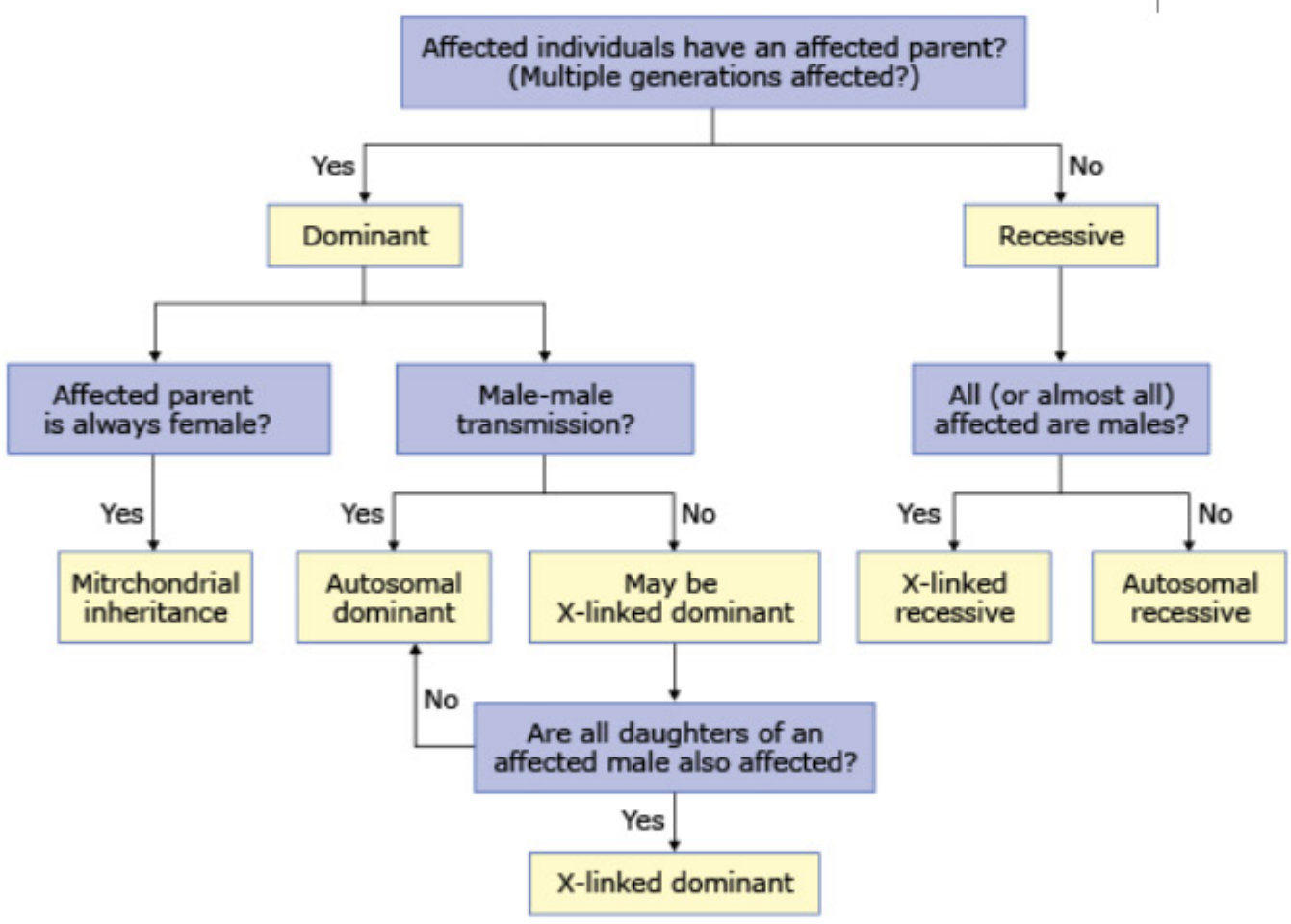
Clinical Application

Diseases With Mitochondrial Inheritance

Because of the importance of mitochondria as organelles of cellular respiration and energy production, mutations in mitochondrial DNA affect tissues which are high in energy utilization. For this reason, the diseases are typically neuropathies and/or myopathies. Important examples include:

- Leber hereditary optic neuropathy
- Mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS)
- Myoclonic epilepsy with ragged red muscle fibers

3.6 Summary Pedigree Analysis Algorithm



▲ **Figure 1–3.6** Pedigree Analysis Algorithm

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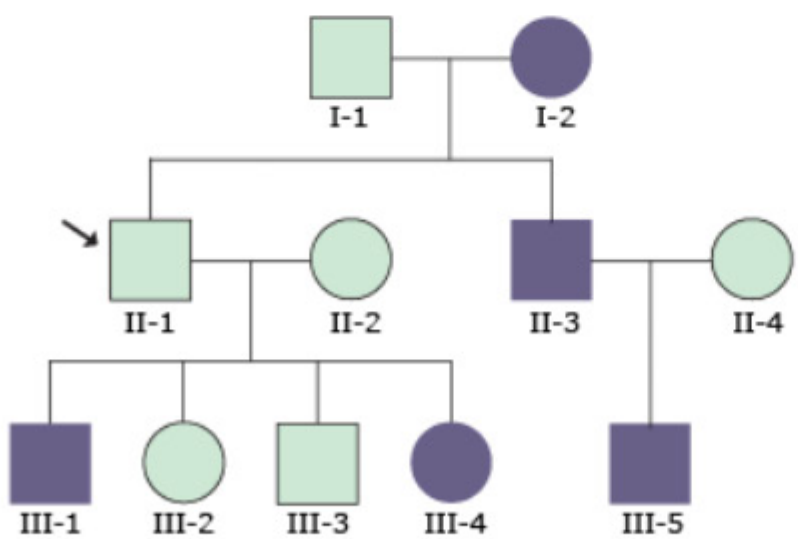
1 Factors Affecting Expression of Single-Gene Diseases

Frequently when examining a pedigree, observations do not exactly fit with one of the classic patterns of inheritance. This discrepancy is due to *genotype-phenotype discordance*, a phrase used to describe situations in which the observed phenotype does not fit with what would be expected based purely on the genotype. Several mechanisms underlie genotype-phenotype discordance.

1.1 Incomplete Penetrance

In some inherited disorders, 100% of individuals with a mutated allele will develop the disease. However, in other instances there is a certain fraction of individuals who never develop disease despite harboring the disease-causing allele. This association between the disease-causing allele and the frequency of individuals who develop the disease is referred to as *penetrance*. For example, if 9 of 10 individuals with a disease-causing allele develop the disease, the penetrance of that allele would be 90%.

Although the mechanisms underlying variable penetrance are complex, it is useful to remember that genes do not exist in isolation, but are part of a complex genome. In this way, genetic background influences whether a person carrying a disease-causing allele develops the disease or remains healthy. Additionally, in certain disorders there may also be environmental factors that mask or promote development of disease. For example, a patient may carry a gene for familial hypercholesterolemia, but if they are on a low-cholesterol diet they may have near-normal cholesterol levels.



▲ **Figure 2–1.1** Incomplete Penetrance

USMLE® Key Concepts

For Step 1, you must be able to:

- Explain inheritance patterns that deviate from the classic single-gene model.
- Describe the genetic mechanisms that cause variability in genetic expression.
- Identify diseases in which incomplete penetrance, variable expression, pleiotropy, locus heterogeneity, delayed age of onset, anticipation, and imprinting occur.

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The penetrance of a disease-causing mutation is evaluated in large numbers of pedigrees by calculating the percentage of individuals who are known to have the disease genotype yet do not display the disease. Penetrance must be considered when predicting recurrence risk in diseases with variable penetrance. For example, if the penetrance of the disease diagrammed in Figure 2–1.1 was determined to be 90%, then the recurrence risk for individuals II-3 and II-4 to have another affected child would be 50% times 90%, or 45%.

1.2 Variable Expression

Variable expression describes the extent to which a disease-causing allele affects an individual. Most genetic diseases vary in the degree of phenotypic expression: some individuals will be severely affected, others less so. This can be due to a variety of factors.

1.2.1 Environmental Factors

Two individuals with identical genotypes may express disease differently for environmental reasons. For example, patients with xeroderma pigmentosum will have much more serious disease if they are exposed to ultraviolet light, and female patients with hereditary hemochromatosis will have less severe disease because of their menstrual loss of blood.

1.2.2 Allelic Heterogeneity

Different mutations in the same disease-causing locus may cause differences in phenotypic expression. For example, hemophilia may be caused by several different mutations that vary in the alteration of the factor VIII gene product. Missense mutations will cause less severe disease than nonsense mutations, which cause the production of truncated, nonfunctional molecules.

1.2.3 Heteroplasmy

In mitochondrial inheritance, the presence of multiple populations of mitochondria, which either possess the mutation or the normal allele, can affect the degree of disease expression.

1.2.4 Modifier Loci

Individuals with disease-causing alleles may have other genes that either enhance or mask the effects of the disease-causing allele.

1.3 Mosaicism

Mosaicism refers to the process by which an organism can have two or more populations of cells within the body with slightly different genotypes. Mosaicism can arise in several ways. Consider the case of X chromosome inactivation already discussed. Random inactivation of X chromosomes in different cells of the blastocyst results in two populations of cells—one with the paternal X chromosome inactivated and one with the maternal X chromosome inactivated. A similar process can occur if a new mutation in the genome arises during embryogenesis—the mutation will only be present in cells derived from the cell in which the mutation occurred, while the other cells of the body will be normal.

In this way, mosaicism of a disease-causing allele may lead to variable severity, tissue-specific effects, or even variable inheritance if gametes are mosaic.

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2 Factors Complicating Inheritance Patterns

2.1 New Mutations

If a genetic disease arises in a family without a previous history of the defect, a new mutation has probably occurred in one of the parental gametes. This would be a common finding in those diseases in which the mortality rate is high or fertility is adversely affected. For example, osteogenesis imperfecta type 2, which results from a defect in collagen synthesis, occurs as a result of new mutations in 100% of cases.

2.2 Locus Heterogeneity

When mutations in different loci can cause the same disease phenotype, it is referred to as *locus heterogeneity*. In osteogenesis imperfecta type 2, for example, the defective phenotype can result from mutations in any one of the three protein chains that make up the triple helix of type 1 collagen. Two of the chains are encoded on chromosome 17 and one on chromosome 7, and mutations in any of these loci can produce phenotypes which are clinically indistinguishable.

2.3 Pleiotropy

Pleiotropy exists when a single genetic defect affects multiple organ systems. As an example, Marfan syndrome is an autosomal dominant disorder caused by mutations in the fibrillin gene. These disease-causing mutations result in individuals who are tall and may develop kyphoscoliosis, eye abnormalities (lens dislocation, retinal detachment), and/or cardiac abnormalities (aortic dissection, mitral valve prolapse). Although these attributes seem quite disparate, fibrillin is a key component of connective tissue in periosteum, perichondrium, aorta, and the suspensory ligament of the eye. The defective molecule is abnormally stretchy, and leads to all the observed features of the disease.

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▲ **Figure 2–2.3** Marfan Syndrome

2.4 Delayed Age of Onset

If a genetic disease is not manifested until later in life, this can complicate the interpretation of a pedigree. Huntington disease is an example of an autosomal dominant disease with delayed age of onset. The defective huntingtin gene is a gain-of-function mutation which causes the buildup of toxic protein aggregates in neurons, causing their death. Most patients develop their first symptoms in the third or fourth decade of life, so it can be difficult to distinguish an unaffected member of a pedigree from one who has the disease allele but has not yet developed symptoms.

Clinical Application

Diseases With Delayed Age of Onset

- Acute intermittent porphyria
- Familial breast cancer
- Hemochromatosis
- Huntington disease

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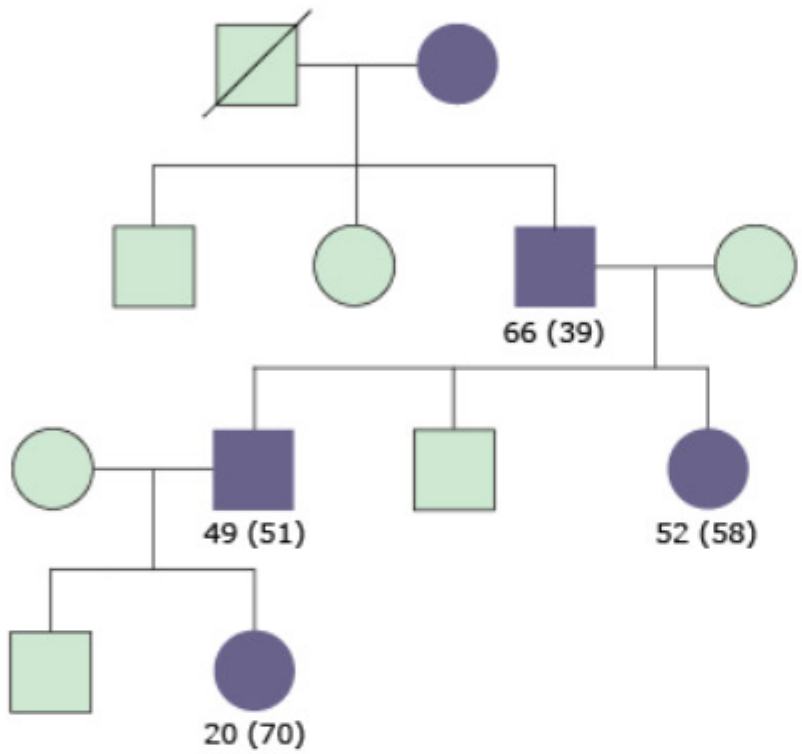
2.5 Anticipation

Anticipation in a pedigree refers to the case in which the disease phenotype is observed earlier in each sequential generation. This is a common observation in diseases that are attributed to trinucleotide repeat expansions in or near a coding gene. Normal phenotypes will have a small number of repeats, which may then become expanded as they are passed to offspring. At some point, a pre-mutation can be expanded to a point that symptoms are observed. The age of onset is correlated with the number of repeats, so as the repeats expand more and more through the generations, onset of disease symptoms occurs earlier and earlier.



Numbers under pedigree symbols identify age of onset (CAG repeats).

▲ **Figure 2–2.5A** Anticipation

▼ **Table 2–2.5** Anticipation: Diseases of Trinucleotide Repeat Expansion

Disease	Repeat	Symptoms
Fragile X syndrome (X-linked dominant)	CGG, 5'UTR	• Intellectual disability • Attention deficit disorder (females) • Large ears and jaw • Postpubertal macroorchidism
Friedreich ataxia (autosomal recessive)	GAA, Intron 1	• Areflexia • Axonal sensory neuropathy • Gait and limb ataxia • Hypertrophic cardiomyopathy • Kyphoscoliosis
Huntington disease (autosomal dominant)	CAG, 5' coding	• Chorea • Emotional lability • Cognitive impairment • Death 10–15 years after onset
Myotonic dystrophy (autosomal dominant)	CTG, 3' UTR	• Cardiac arrhythmia • Muscle loss • Testicular atrophy • Frontal baldness • Cataracts

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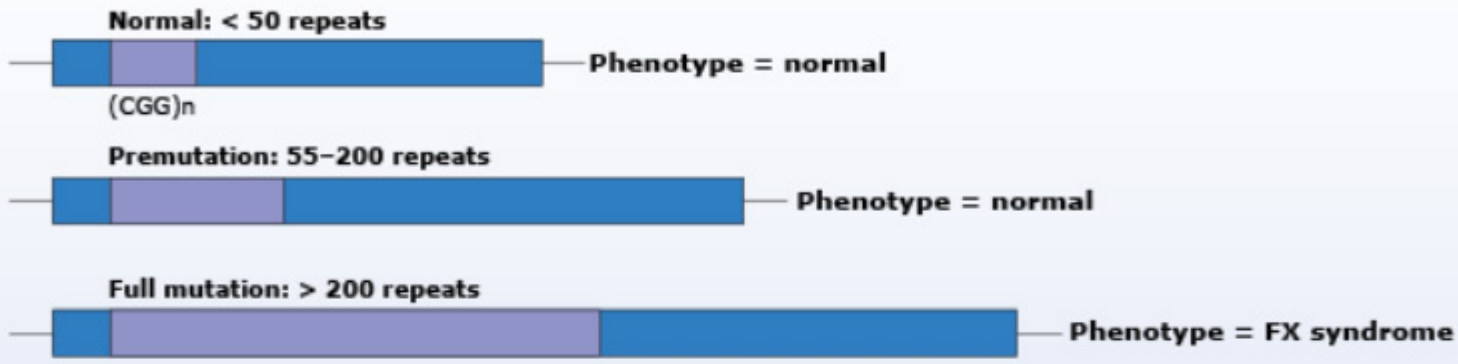
Clinical Application

CGG Repeat Mutation in Fragile X Syndrome

This disease is an X-linked dominant CGG trinucleotide repeat in the FMR1 gene on the X chromosome. Normal individuals have 6–54 copies. A repeat of 50–200 copies will produce an asymptomatic pre-mutation. Individuals with 200–1300 repeats have the disease phenotype.



▲ **Figure 2–2.5B** CGG Repeat Mutations

2.6 Imprinting

Imprinting is a phenomenon by which certain genes are expressed in a parent-of-origin-specific manner. Imprinted alleles are silenced (by methylation) such that the genes are either expressed only from the non-imprinted allele inherited from the mother, or in other instances from the non-imprinted allele inherited from the father. Rarely, the transcriptionally active gene can be deleted from the chromosome during gametogenesis. This leaves the child with no active gene at this locus: One copy was imprinted and thereby inactive, and the other copy was deleted by mutation. In Prader-Willi syndrome, deletion of an imprinted locus mapping to 15q11-13 from the paternal chromosome includes the gene SNRPN, which encodes a protein for mRNA splicing. Children with Prader-Willi syndrome have moderate levels of intellectual disability, along with hypogonadism and obesity.

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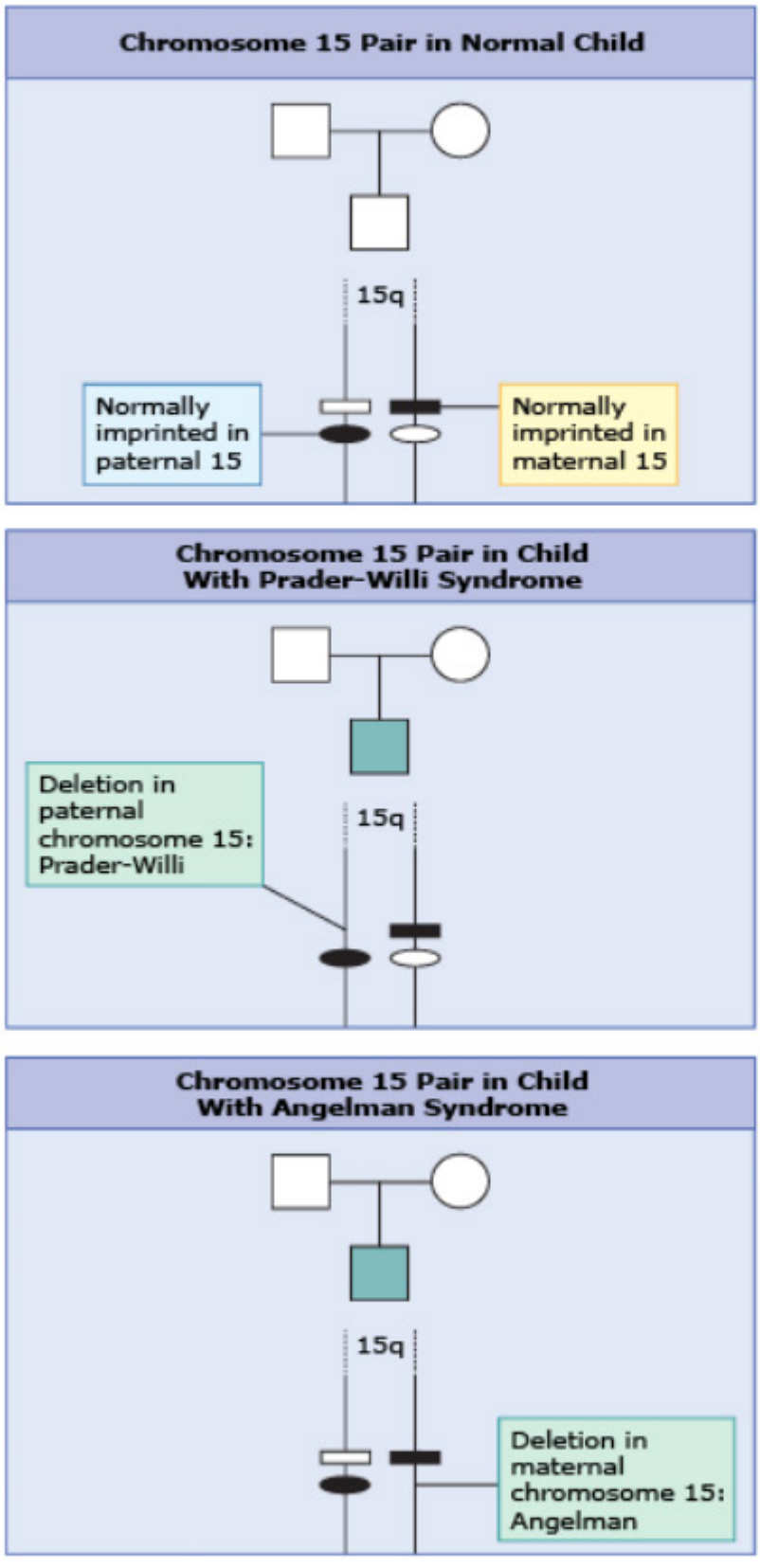
Angelman syndrome results if there is a deletion of 15q11-13 from the maternal chromosome. This area contains UBE3A, which is a gene involved in the ubiquitin pathway that is normally expressed in the mother and silenced in the father. Children with Angelman syndrome have severe intellectual disability, ataxia, and seizures. This disorder is also known as the "happy puppet" syndrome due to the characteristic movements affected children display as a consequence of ataxia.



▲ **Figure 2–2.6A** Imprinting

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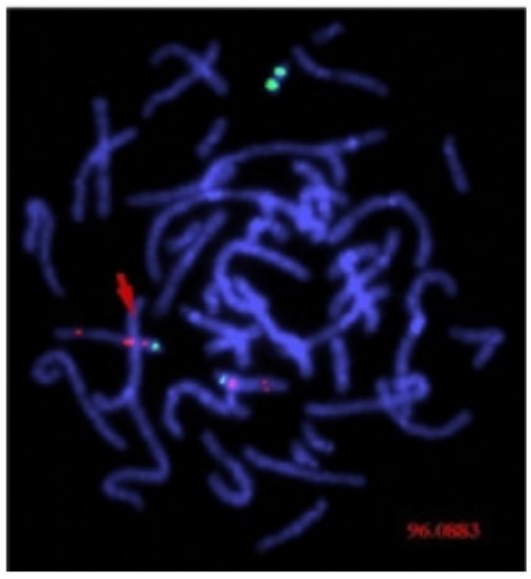
▲ **Figure 2–2.6B**
Prader-Willi Syndrome



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▲ **Figure 2–2.6C**
Chromosomal Deletion

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▲ **Figure 2–2.6D**
Angelman Syndrome

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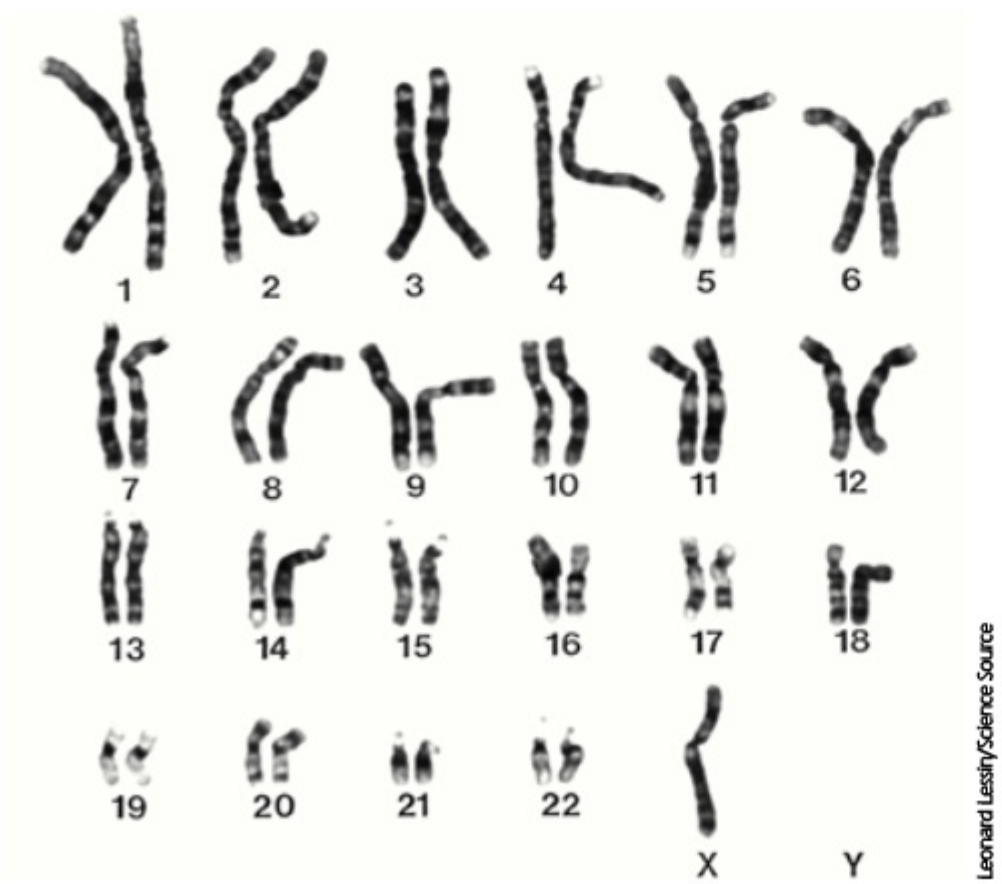
1 Overview of Cytogenetics

Cytogenetics is the study of microscopically observable changes in chromosomes. These may involve changes in the number of chromosomes or alterations in the structure of the chromosomes. Chromosome abnormalities are the leading cause of intellectual disability (mental retardation). These abnormalities are also the major cause of pregnancy loss.

2 Chromosomal Morphology and Nomenclature

2.1 Karyotype

A *karyotype* is an ordered photographic display of the 23 human chromosomes observed microscopically during the metaphase stage of mitosis when the chromosomes are maximally condensed. A *karyogram* is a drawing of each chromosome. Chromosomes are ordered and numbered in order of size, with the largest chromosome being No. 1 and the smallest, No. 22. The sex chromosomes are placed in the lower right of the karyotype.



▲ **Figure 3–2.1** Karyotype

USMLE® Key Concepts

For Step 1, you must be able to:

- Read and interpret karyotypes.
- Identify the common diseases associated with autosomal and sex chromosome aneuploidies.
- Explain the role of nondisjunction in the creation of monosomies and trisomies.
- Describe the formation of reciprocal and Robertsonian translocations and their effects on gametogenesis.

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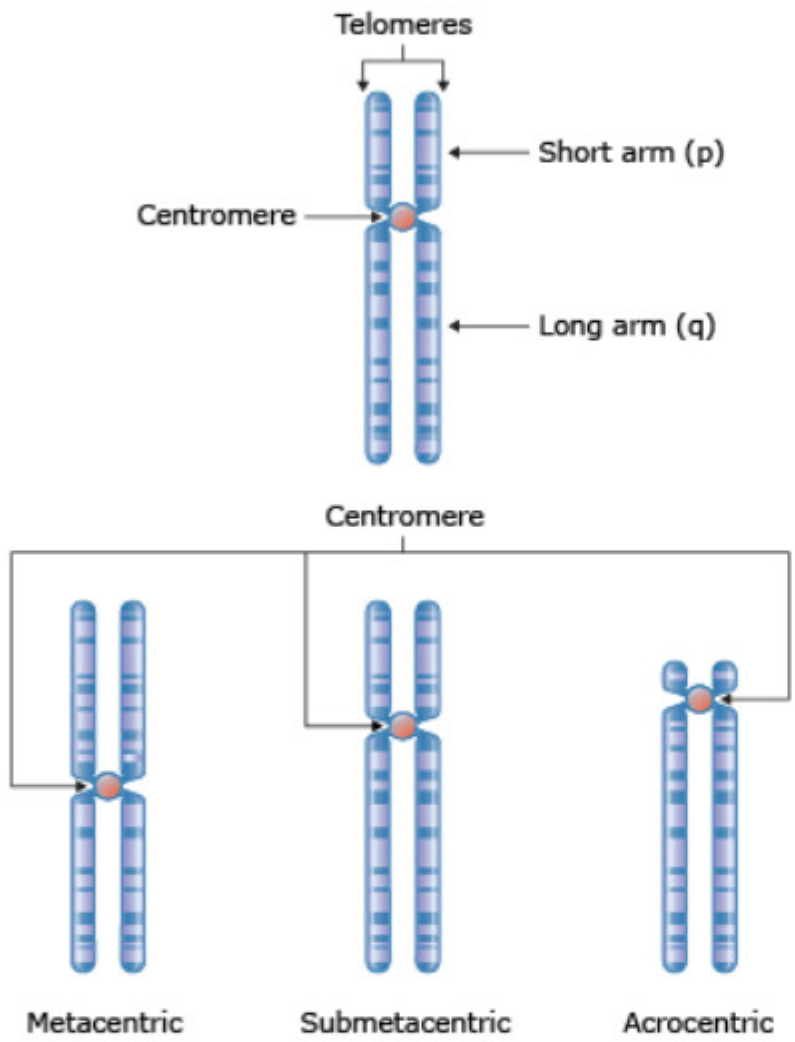
2.2 Chromosome Banding

G-banding, or Giemsa banding, is a technique to visualize the chromosomes in a karyotype unambiguously. This reveals a pattern of light and dark bands which can then be used to identify large deletions or other morphologic abnormalities of the chromosomes.

2.3 Chromosome and Karyotype Nomenclature

Each mitotic chromosome contains a centromere and two sister chromatids.



▲ **Figure 3–2.3** Chromosome Types

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▼ **Table 3–2.3** Chromosome and Karyotype Nomenclature

Term	Meaning
Metacentric chromosome	Chromosomes in which the centromere is nearly central, and the arms are of roughly equal length
Submetacentric chromosome	Chromosomes in which the centromere is offset from the center, so the arms are of different length
Acrocentric chromosome	Chromosomes in which the centromere is far to one end, with one arm bearing little genetic information
Telomeres	Tips of the chromosomes
Autosomes	Chromosomes 1 to 22
X, Y	Sex chromosomes
(+) or (−)	When placed in front of a chromosome number, it implies that the chromosome is extra or missing, respectively
p	"Petite"—the short arm of the chromosome
q	Long arm of the chromosome
t	Translocation
del	Deletion

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3 Abnormalities of Chromosome Number

3.1 Euploidy

A human cell with some multiple of 23 chromosomes is said to be *euploid*. Gametes (sperm and egg) have one copy of each chromosome and are said to be *haploid*. Somatic cells are generally *diploid*, having two copies of each chromosome, a total of 46. *Triploid* conceptions, in which each cell contains three copies of each chromosome for a total of 69 chromosomes, are generally lost prenatally. Only a few cases of *tetraploidy* (92 total chromosomes) have ever been observed, and the children do not survive.

3.2 Aneuploidy

Aneuploidy is defined as a deviation from the euploid number by either the gain or loss of a specific chromosome. In *monosomy* there is loss of a chromosome, leaving only one copy of that pair available, and in *trisomy* there is the gain of a chromosome, giving three copies of one chromosome to each cell.

3.2.1 Autosomal Aneuploidies

There are no autosomal monoploidies that are consistent with a live birth. Trisomy is the most common genetic cause of spontaneous abortion. Only three autosomal trisomies can produce live births.

Trisomy 21 (Down Syndrome) The most common condition resulting from an autosomal trisomy, Down syndrome occurs in 1/800 live births. Specifically, it results from trisomy of chromosome 21 (47,+21) and leads to intellectual disability (IQ 20–70) with specific physical features, including short stature, depressed nasal bridge, upslanting palpebral fissures, and epicanthal folds. Other associated conditions include congenital heart defects, early onset dementia, and increased risk of leukemia.

Lauren Shear/Science Source

▲ **Figure 3–3.2A** Down Syndrome

Leonard Lessin/Science Source

▲ **Figure 3–3.2B** Down Syndrome Karyotype

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Trisomy 18 (Edwards Syndrome) Affecting 1/8,000 live births, trisomy 18 (47,+18) causes Edwards syndrome. It leads to abnormal features, including low-set ears, prominent occiput, micrognathia, flexion of the fingers, and rocker-bottom feet. Edwards syndrome is also associated with intellectual disability and congenital heart disease. Affected children typically die within the first year of life.

Courtesy of the Centers for Disease Control

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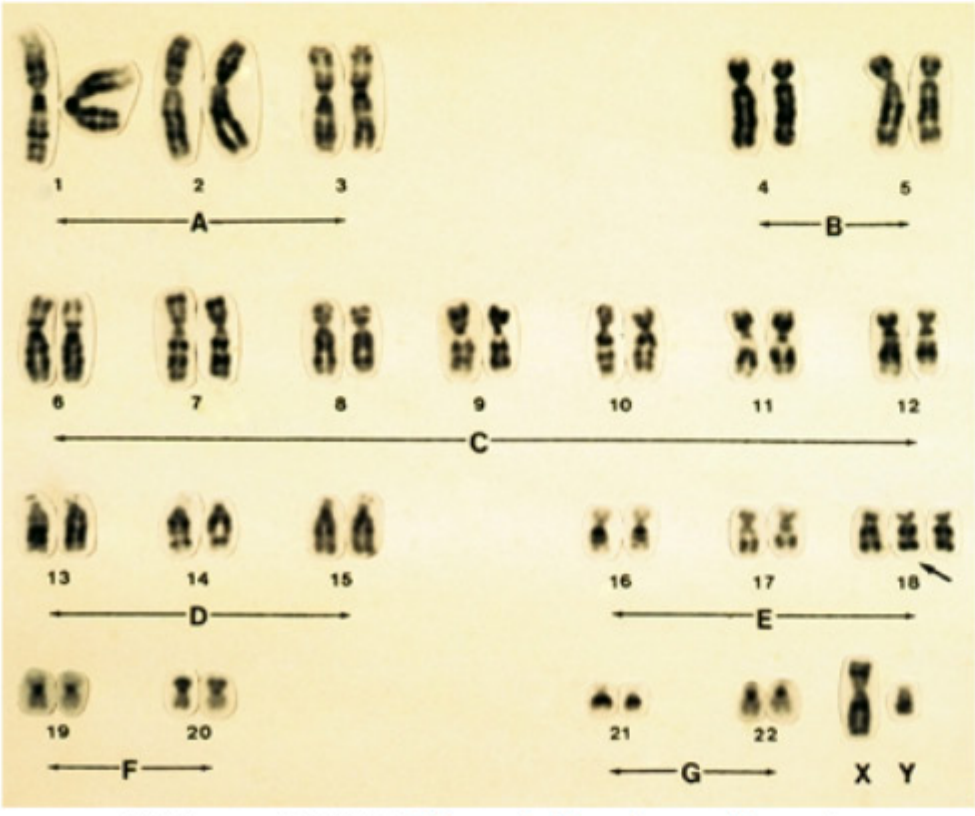
▲ **Figure 3–3.2C** Edwards Syndrome



Richard J. Greery/Science Source

▲ **Figure 3–3.2D** Edwards Syndrome Karyotype

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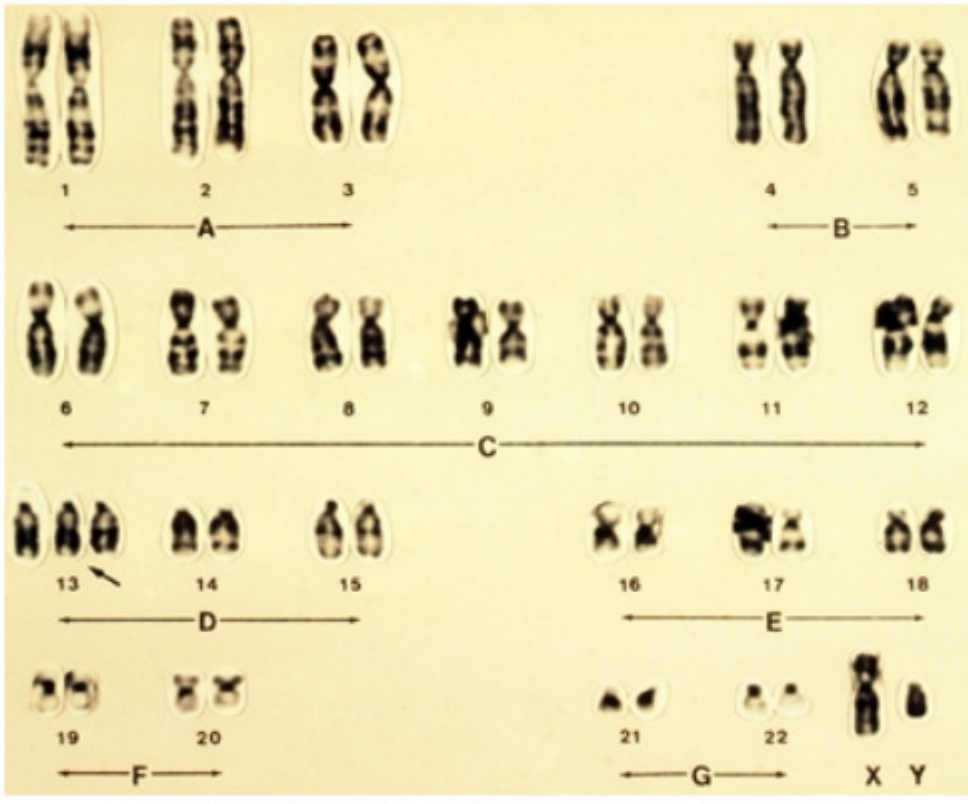
Trisomy 13 (Patau Syndrome) The most severe of the autosomal trisomies, trisomy 13 (47,+13) results in Patau syndrome. Approximately 1/6,000 live births are affected and children have intellectual disability with microphthalmia, microcephaly, cleft lip and palate, polydactyly and, frequently, congenital heart disease. These children also usually die within the first year.

Ralph C. Eagle, Jr./Science Source

Biophoto Associates/Science Source

▲ **Figure 3–3.2E** Patau Syndrome



Richard J. Green/Science Source

▲ **Figure 3–3.2F** Patau Syndrome Karyotype

3.2.2 Sex Chromosome Aneuploidies

Sex chromosome aneuploidies are more common and have less severe consequences than autosomal aneuploidies. At least one X chromosome is required for survival. If there is more than one X chromosome, all but one will become a Barr body in the cells. With minor exceptions, the presence of the Y chromosome defines the male phenotype.

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Turner Syndrome The only monosomy leading to a viable offspring, Turner syndrome (45,X) results from a lack of one of the sex chromosomes. Females with Turner syndrome have short stature with webbed necks and often cystic hygromas. They also may present with ovarian dysgenesis leading to primary amenorrhea.

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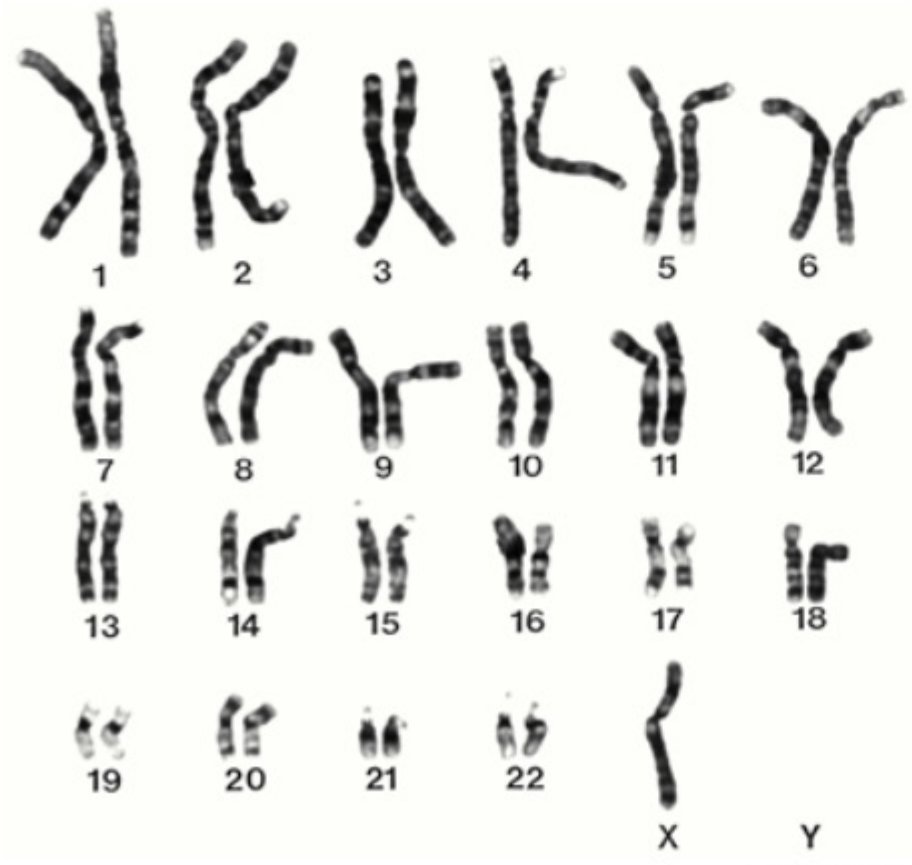
▲ **Figure 3–3.2G** Turner Syndrome



SPL/Science Source

◀ **Figure 3–3.2H** Turner Syndrome Karyotype

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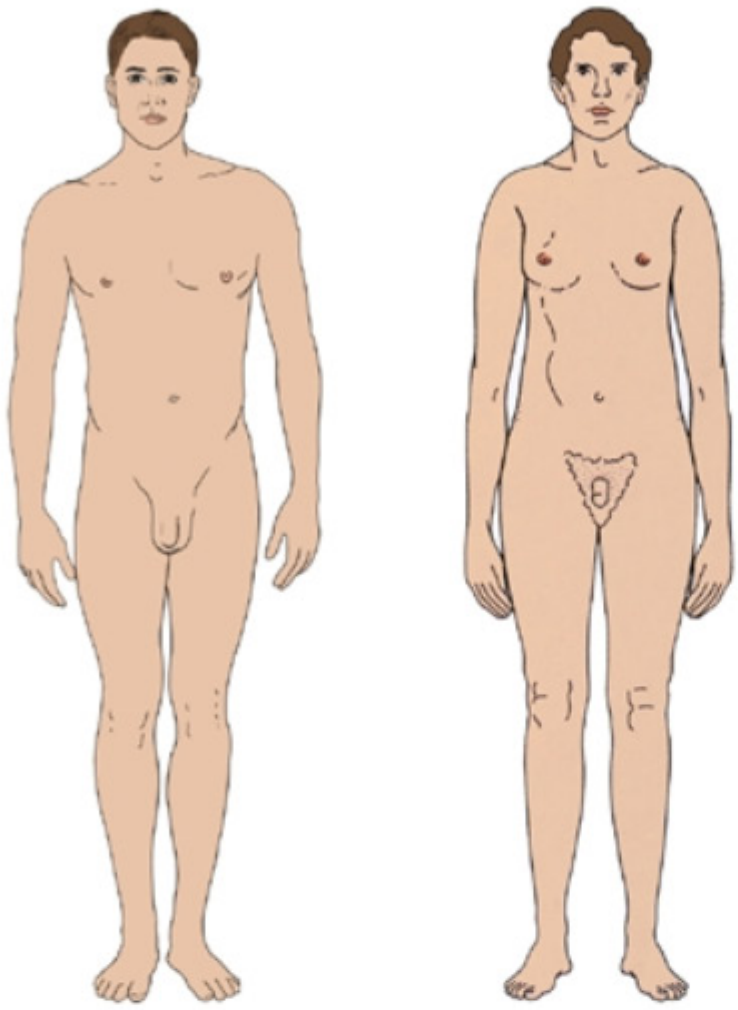
Klinefelter Syndrome This trisomy leads to males with an extra X chromosome (47,XXY). Individuals affected by Klinefelter syndrome typically have tall stature with long arms and legs. They also may have testicular atrophy, gynecomastia, and a female distribution of body hair.



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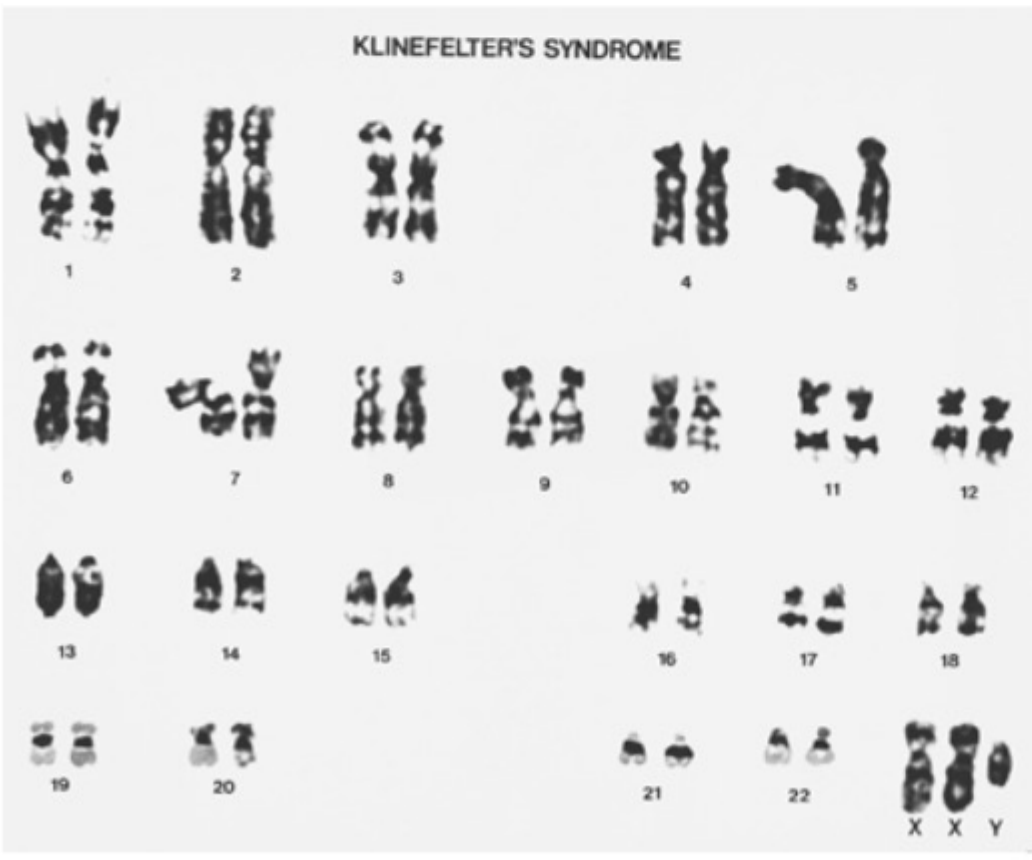
▲ **Figure 3–3.2I** Klinefelter Syndrome: Normal (*Left*) and Extra X Chromosome (*Right*)



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▲ **Figure 3–3.2J** Klinefelter Syndrome Karyotype

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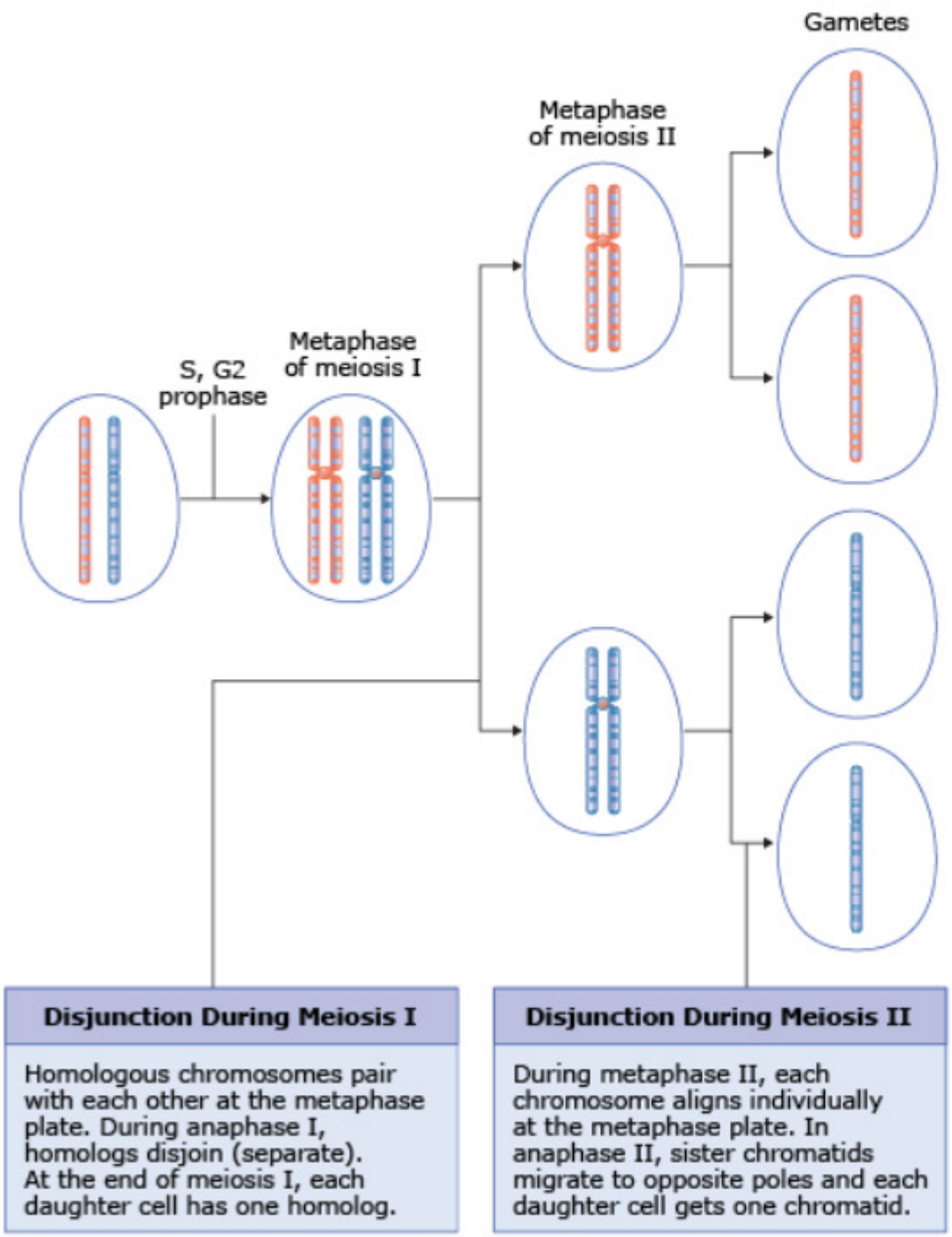
3.3 Mechanisms Causing Aneuploidies

Aneuploidy typically arises because of the loss or gain of a chromosome due to errors in meiosis. The most common type of error that occurs is called nondisjunction. Nondisjunction is a failure of chromosomes to separate during meiosis and can occur during either meiosis I or meiosis II.

3.3.1 Normal (Disjunction) in Meiosis

During meiosis I, DNA is replicated and homologous chromosomes, each made up of two sister chromatids, are arranged and then segregated into two daughter cells. During meiosis II, sister chromatids are separated.



▲ **Figure 3–3.3A** Disjunction in Meiosis

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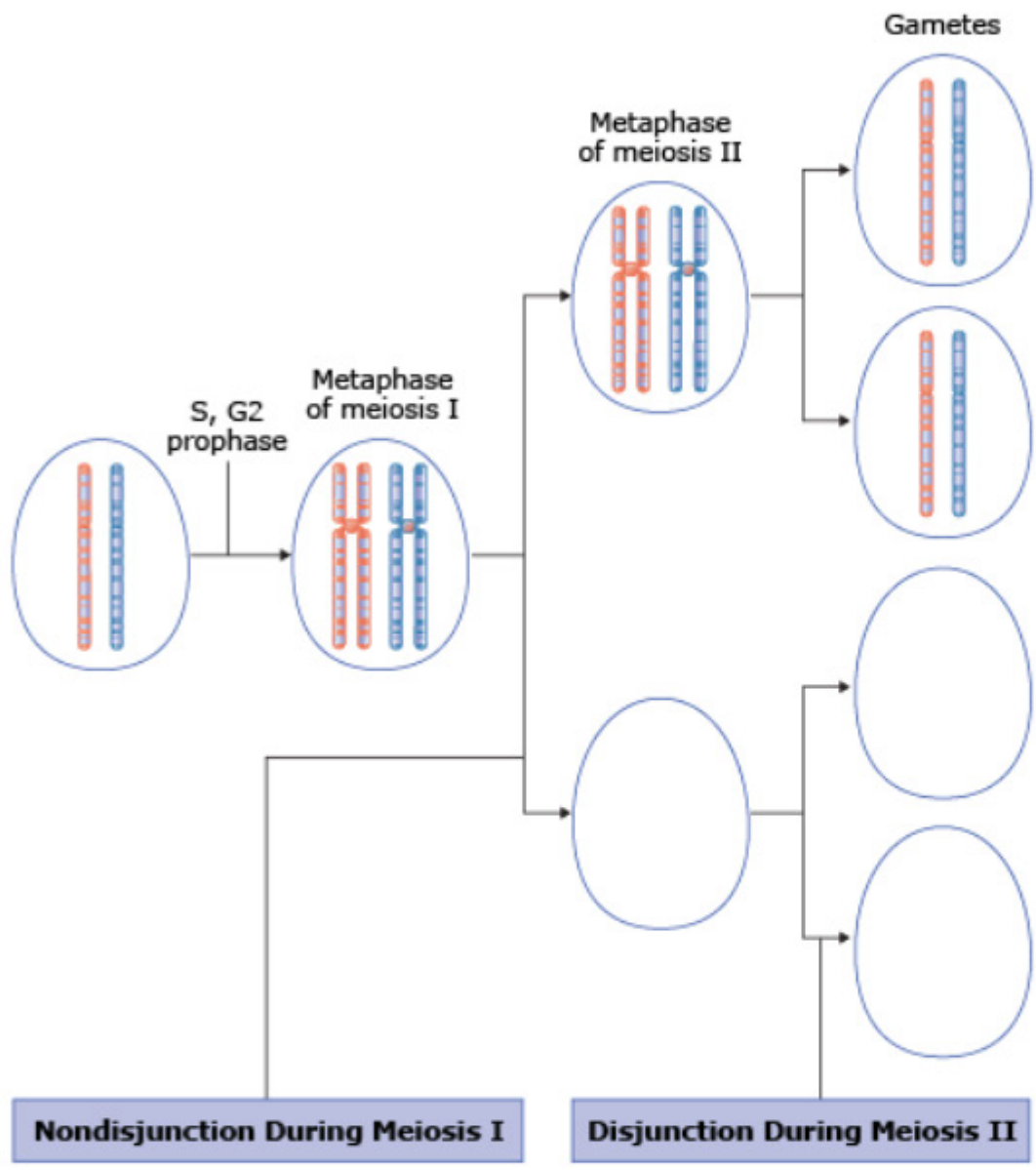
3.3.2 Nondisjunction in Meiosis I

Failure of separation between a pair of homologous chromosomes in meiosis I results in one daughter cell with an extra chromosome (containing a pair of sister chromatids) and one daughter cell lacking a chromosome. During meiosis II, the sister chromatids divide. The cell with the extra chromosome produces two gametes with an extra chromosome each (n = 24), while the cell lacking that chromosome produces two gametes lacking a chromosome (n = 22).



▲ **Figure 3–3.3B** Nondisjunction in Meiosis I

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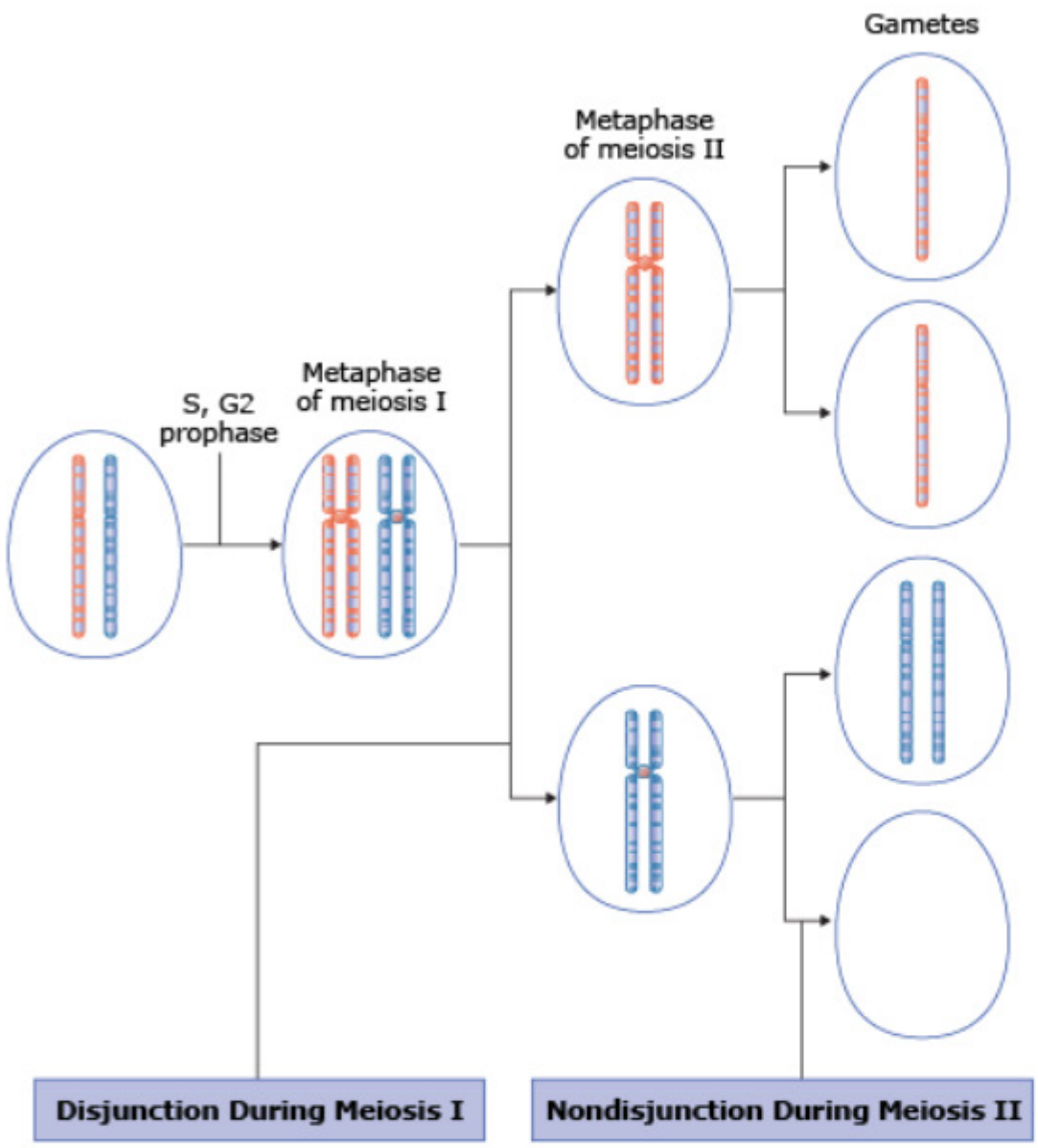
3.3.3 Nondisjunction in Meiosis II

In this case, one of the daughter cells produced during meiosis I segregates its sister chromatids normally and two haploid gametes are produced (n = 23). In the other daughter cell, one pair of sister chromatids fails to separate, resulting in one gamete with an extra chromosome (n = 24) and one gamete lacking a chromosome (n = 22).



▲ **Figure 3–3.3C** Nondisjunction in Meiosis II

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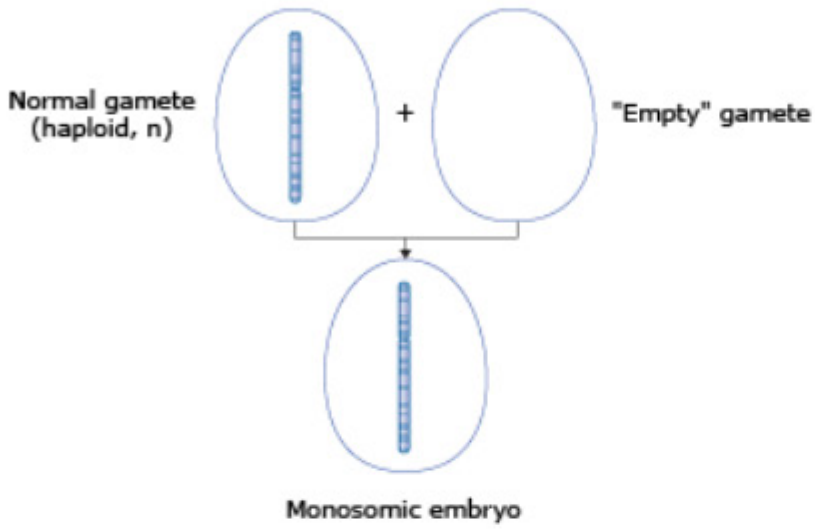
3.3.4 Consequences of Meiotic Nondisjunction

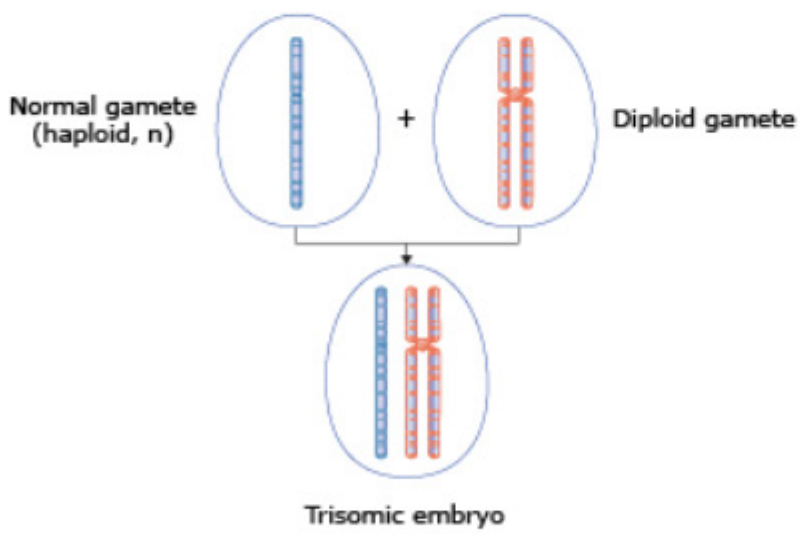
Following the process of nondisjunction, aneuploid gametes result. If one of these aneuploid gametes fuses with another gamete during fertilization, monosomy or trisomy will result.

Monosomy The combination of a normal gamete (n = 23) with an aneuploid gamete lacking a chromosome (n = 22) will lead to a zygote missing one chromosome of a pair (either the paternal or maternal). This situation is termed monosomy and the zygote has 45 chromosomes in total.



▲ **Figure 3–3.3D** Monosomy

Trisomy The combination of a normal gamete (n = 23) with an aneuploid gamete with an extra chromosome (n = 24) will lead to a zygote with three chromosomes of a pair. This situation is termed trisomy and the zygote has 47 chromosomes in total.



▲ **Figure 3–3.3E** Trisomy

Causes of Down Syndrome

Trisomy 21, like most trisomies, is associated with advanced maternal age due to higher rates of meiotic nondisjunction. Eighty percent of maternal nondisjunction events occur at meiosis I, with the remaining 20% occurring at meiosis II. There is no effect of paternal age on the incidence of Down syndrome. In 4% of cases, Down syndrome may be familial, resulting from a Robertsonian translocation (which will be covered later). Somatic mosaicism, as a result of mitotic nondisjunction during embryogenesis, causes ~1% of cases of Down syndrome.

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3.4 Other Consequences of Nondisjunction: Uniparental Disomy

In rare cases, both copies of a given chromosome are contributed by a single parent, a condition known as *uniparental disomy*. If the chromosome in question has imprinted regions, this may result in abnormal phenotype. For example, 25% to 30% of all Prader-Willi cases result from maternal uniparental disomy of chromosome 15. A smaller proportion of Angelman syndrome cases result from paternal uniparental disomy of the same chromosome.

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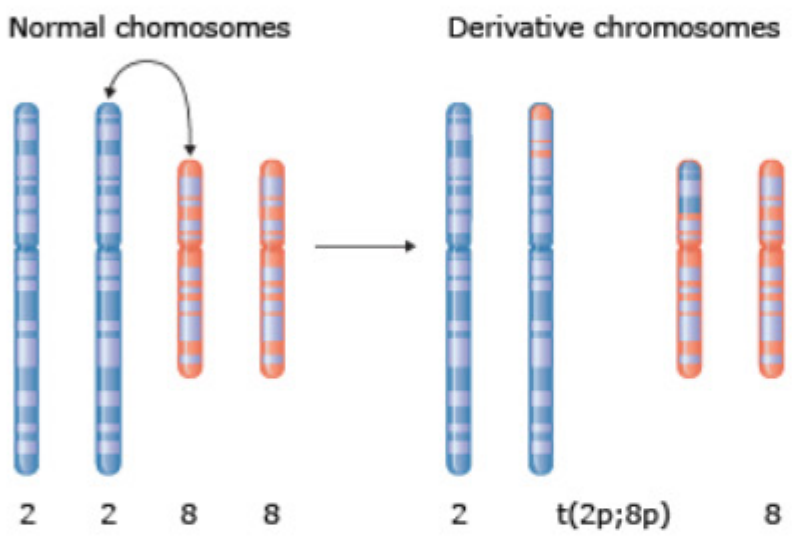
4 Structural Abnormalities of Chromosomes

4.1 Translocations

Translocation involves the physical movement of genetic material on one chromosome to another. There are two major types of translocations, reciprocal and Robertsonian.

4.1.1 Reciprocal Translocation

Reciprocal translocations are those in which genetic material is swapped between two chromosomes. Typically, reciprocal translocations involve an even substitution of genetic material from one chromosome to another. There is no loss of genetic material and the translocation is referred to as "balanced."



▲ **Figure 3–4.1A** Reciprocal Translocation

Offspring of individuals with a balanced translocation are at risk for developing a partial trisomy or partial monosomy. During meiosis, when homologous chromosomes are paired and then segregated into daughter cells, if one of these chromosomes holds a translocation, that translocated segment will be improperly paired and segregated. As a result, one gamete will contain an extra portion of the translocated chromosome and one will lack that portion of chromosome. Following fertilization, this results in what is termed a partial trisomy (for the zygote with the translocated portion) or a partial monosomy (for the zygote lacking the translocated portion).

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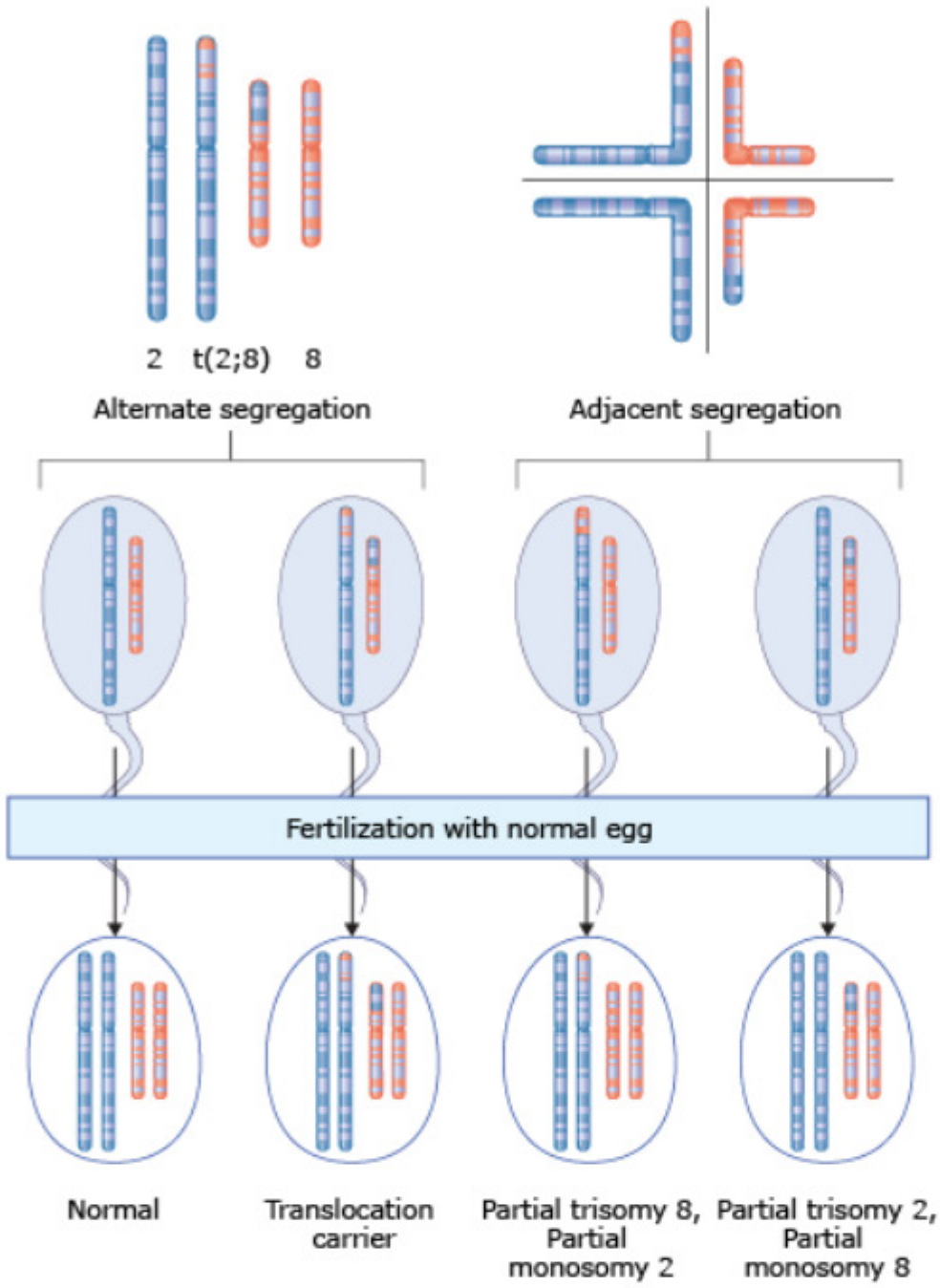
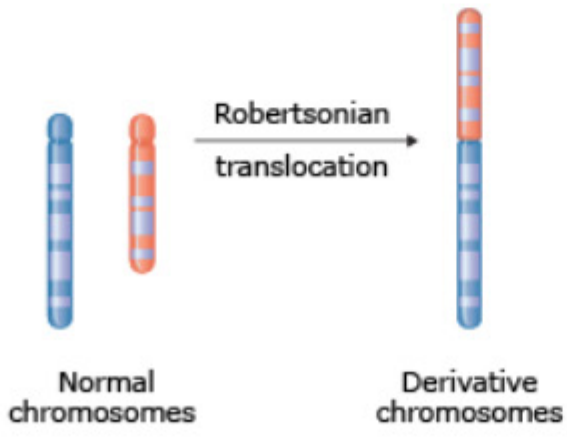


▲ **Figure 3–4.1B** Partial Trisomy and Partial Monosomy

4.1.2 Robertsonian Translocation

A Robertsonian translocation results from the fusion of two acrocentric chromosomes. Such chromosomes have small p arms and these usually are lost in the process of translocation. Fortunately, because the lost p arms contain little genetic information, individuals harboring a Robertsonian translocation usually have no phenotypic consequences.



▲ **Figure 3–4.1C** Robertsonian Translocation

Connection to Pathology

Reciprocal Translocations in Somatic Cells

Translocations in somatic cells often are associated with malignant transformation. The most famous of these, the Philadelphia Chromosome, involves a reciprocal translocation of the long arms of chromosomes 9 and 22. This alters the activity of the abl protooncogene, and in hematopoietic cells, results in the production of chronic myelogenous leukemia. Other translocations involved in malignant transformation include:

- t(8;14): Burkitt lymphoma, c-myc
- t(11;14) : Mantle cell lymphoma, cyclin D
- t(14;18): Follicular lymphoma, bcl-2
- t(15;17) : Acute myelogeous leukemia, retinoid receptor-α

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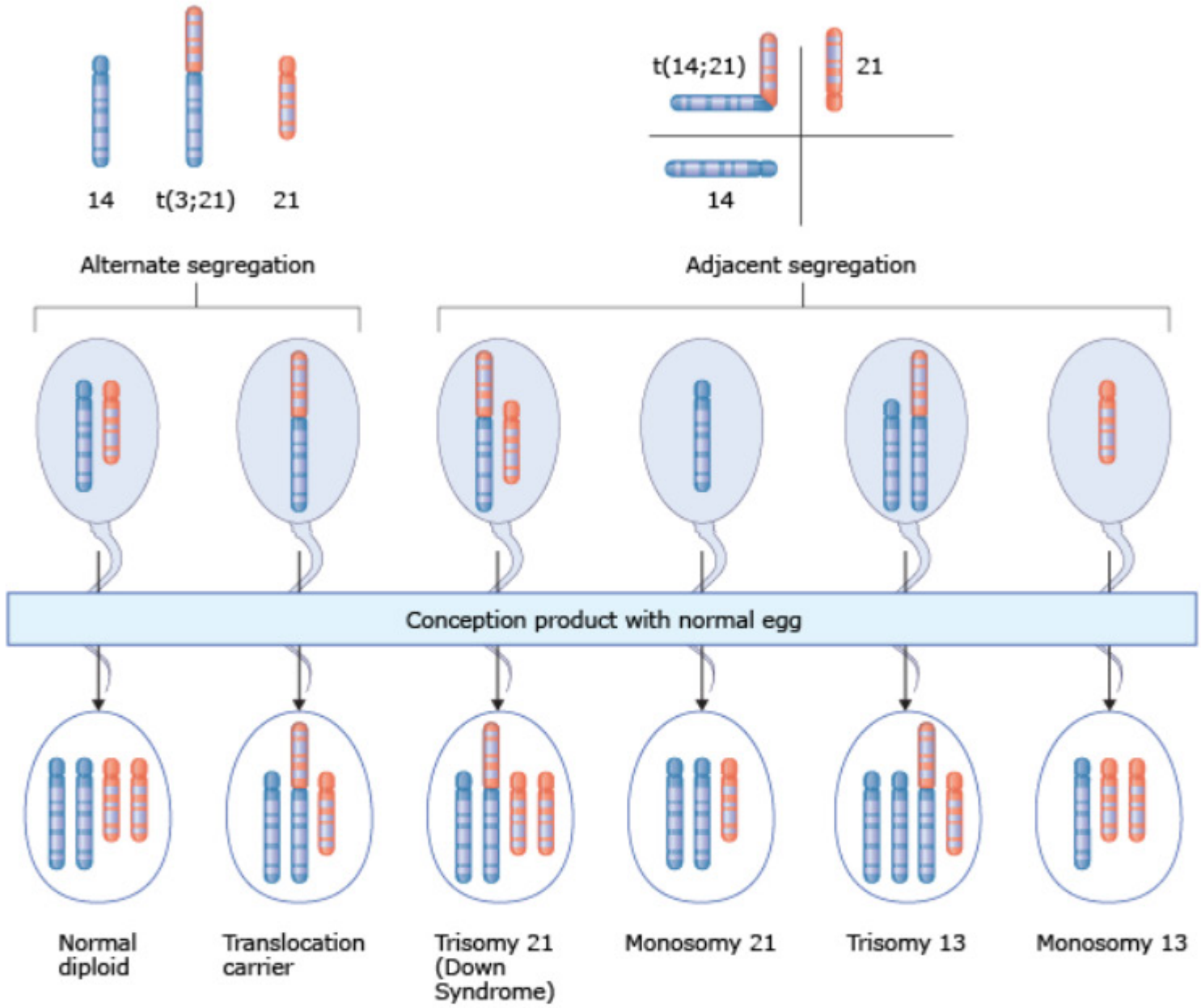
Individuals with Robertsonian translocations also are at risk for generating offspring with partial monosomy or partial trisomy. In fact, familial Down syndrome is a consequence of a Robertsonian translocation involving chromosome 14 and chromosome 21. A subset of offspring from a parent with this translocation will have partial trisomy 21, leading to the disease as depicted in Figure 3–4.1D.



▲ **Figure 3–4.1D** Robertsonian Translocation and Trisomy 21

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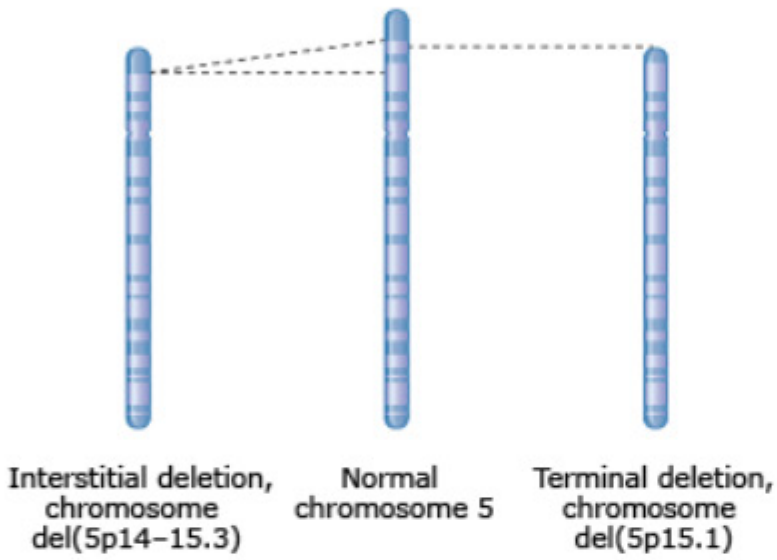
4.2 Deletions and Microdeletions

Deletions involve the loss of genetic material—from portions of genes to entire chromosomes. Three well-known deletion syndromes are Angelman and Prader-Willi, which have been previously described, and cri-du-chat.

4.2.1 Cri-du-Chat Syndrome

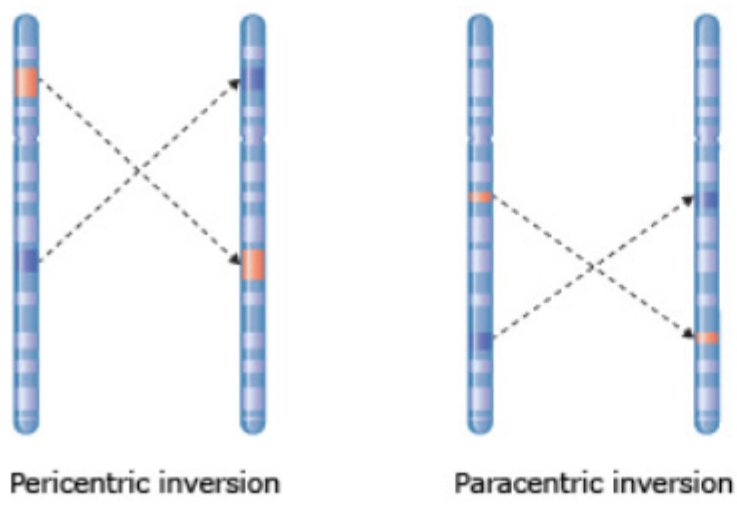
Cri-du-chat syndrome is a rare disorder caused by deletion of the short arm of chromosome 5 [46,del(5p)]. It affects 1/25,000 to 1/50,000 live births and leads to microcephaly and severe intellectual disability. Children with cri-du-chat have a characteristic high-pitched cry for which the syndrome is named. Cardiac abnormalities are common and these children generally do not survive to adulthood.



▲ **Figure 3–4.2** Cri-du-Chat Syndrome

4.3 Inversions

An inversion is another chromosomal abnormality that may occur during meiosis. Specifically, during the process of meiotic recombination, portions of a chromosome may be "flipped" or inverted. The inversion may be small or large. Those that involve the centromere are referred to as *pericentric*, and those that are confined to the ends of the chromosome and do not involve the centromere are referred to as *paracentric*. The inversion results in the physical rearrangement of genetic material and, as such, inversions also can lead to the development of disease.



▲ **Figure 3–4.3** Pericentric and Paracentric Inversion

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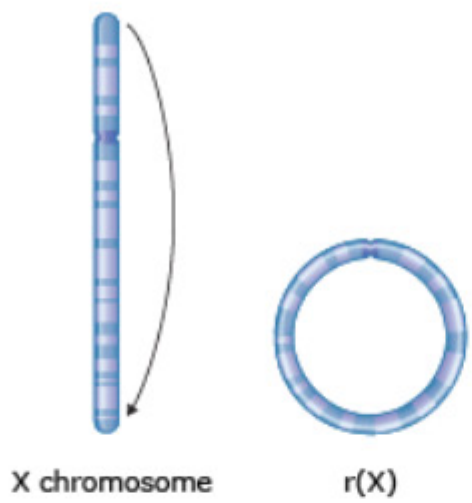
4.4 Ring Chromosome

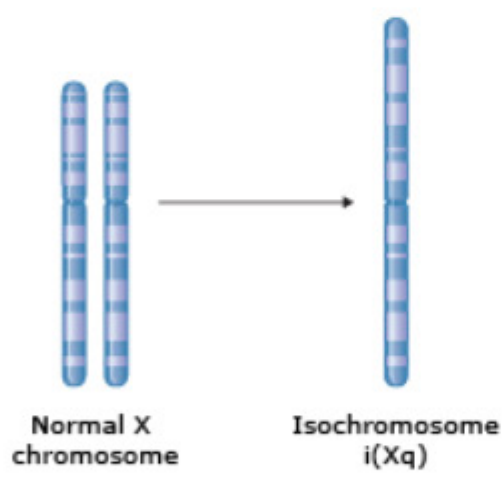
When a deletion occurs on both tips of a chromosome and the remaining ends fuse, a ring chromosome results. A female with this condition would be 46,X,r(X). Because ring chromosomes pair poorly during meiosis, they are often lost and monosomies result.



▲ **Figure 3–4.4** Ring Chromosome

4.5 Isochromosome

If a chromosome divides along a plane that is perpendicular to the normal axis of division, an *isochromosome* is created that has two copies of one arm and none of the other. Autosomal isochromosomes are, therefore, lethal because they result in monosomy for the arm that has been lost. X chromosome isochromosomes are noted as 46,X,i(Xq).



▲ **Figure 3–4.5** Isochromosome

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1 Overview of Population Genetics

The study of genetic variation in a population is called *population genetics*. These studies allow us to evaluate the roles of evolutionary factors such as natural selection, genetic drift, and gene flow in changing gene frequencies in human populations.

1.1 Genotype Frequencies

The *genotype frequency* is the proportion of a given genotype at a specific locus in the population: In other words, the *frequency* (f) of homozygous for one allele, heterozygous, and homozygous for the alternative allele: f(AA), f(Aa), and f(aa).

Because these are the only possible genotypes, it naturally follows that:

$$f(AA) + f(Aa) + f(aa) = 1$$

If a population was assayed for the presence of a particular genetic polymorphism at a specific locus, and it was determined that 48/100 individuals possessed the AA genotype, 44/100 possessed the Aa genotype, and 8/100 possessed the aa genotype, then their genotype frequencies would be expressed as:

- AA = 0.48
- Aa = 0.44
- aa = 0.08

1.2 Allele Frequencies

The allele frequency is the actual number of alleles at that locus on chromosomes. To continue with the example given above:

- The AA genotype has two copies of the A allele.
- The Aa genotype has one copy of the A allele and one of the a allele.
- The aa genotype has two copies of the a allele.

Therefore, to calculate the allele frequency of the A allele in the population cited above, we would take the number of AA individuals (48) and realize that they had two copies (2 × 48); take the number of Aa individuals (44) and realize that they had one copy; and because the number of chromosomes in a diploid population of 100 people would be 200 for that chromosome, the formula becomes:

$$\frac{(2 \times 48) + 44}{200} = 0.7$$

Then, a shortcut for the determination of the allele frequency for "a" becomes 1 − 0.7 = 0.3 because the two allele frequencies added together must always equal 1.

USMLE® Key Concepts

For Step 1, you must be able to:

- Explain the meaning of allele frequency and genotype frequency.
- Estimate genotype frequencies or allele frequencies in recessive, dominant, and sex-linked diseases using the Hardy-Weinberg equation.
- Describe the roles of mutation, natural selection, genetic drift, gene flow, and consanguinity in population genetics.

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2 Hardy-Weinberg Equilibrium

In large populations that are mating at random (with respect to a given allele), there should be a constant and predictable relationship between genotype frequencies and allele frequencies. This is expressed as the *Hardy-Weinberg equilibrium,* and if one knows the inheritance pattern of a specific disease and the frequency of that disease, the equation can be used to calculate the frequency of alleles in that population:

- p = frequency of the normal allele
- q = frequency of the disease allele
- p^2 = frequency of genotype AA
- 2pq = frequency of the heterozygous genotype
- q^2 = frequency of genotype aa

So the Hardy-Weinberg equation results:

$$p^2 + 2pq + q^2 = 1$$

2.1 Determining Genotype Frequencies for Autosomal Recessive Diseases

In a population in which 1% of the individuals have a recessive disease, what percentage of the individuals are asymptomatic carriers of the disease?

In an autosomal recessive disease, asymptomatic carriers will be heterozygotes. Thus, the question is really asking for f(Aa) = 2pq.

To solve this, we start with the information we have, the frequency of diseased individuals, $f(aa) = q^2$.

Calculating f(a) or q:

- $q^2 = 0.01$
- $q = 0.1$

Because q = 0.1, we can determine p and thus f(A):

- $p + q = 1$
- $p = f(A) = 0.9$

Using the last portion of the Hardy-Weinberg equation, we can calculate f(Aa) or 2pq:

$$2pq = 2(0.9)(0.1) = 0.18$$

Thus, 18% of the population are carriers of the disease.

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2.2 Determining Genotype Frequencies for Autosomal Dominant Diseases

In the case of autosomal dominant diseases, the largest number of affected individuals will be heterozygotes, or 2pq. Using the shortcut that the normal alleles in a population far outnumber the disease alleles, we can approximate the value of p as being close to 1, and then the formula becomes 2q.

If the number of diseased individuals in a population is 1/500, then 2q is 1/500, so q^2 is the number of homozygous diseased individuals, or $1/10^6$.

2.3 Determining Genotype Frequencies for Sex-Linked Diseases

Because males are hemizygous for the X chromosome, when applying the Hardy-Weinberg equilibrium to X-linked recessive diseases, q (disease allele frequency) equals the prevalence of affected males. Therefore, if the number of hemophiliac males in a population is 1/10,000, this is the allele frequency q. The prevalence of disease in females is therefore q^2 or 1/100,000,000, and the prevalence of female carriers is 2q or 1/5,000. Once again, the majority of the recessive alleles are hidden in the female heterozygotes, but a significant number are expressed in affected males.

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3 Genetic Variables Affecting Hardy-Weinberg Equilibrium

The Hardy-Weinberg equilibrium works only for populations in which only two alleles are present. Additionally, in order for the equation to function properly, several additional assumptions must hold true. Specifically:

- There must be no new mutations.
- There can be no selection pressure for or against alleles.
- There can be no genetic drift.
- There can be no gene flow in or out of the population.

All of these conditions change the genotype, and thus the allele frequencies, and disturb the Hardy-Weinberg equilibrium.

3.1 New Mutations

Spontaneous mutations will alter the allele frequencies and upset the equilibrium. For example, if new mutations in a gene occurred frequently, the frequency of the q allele would be ever increasing. The odds of someone having the q allele would be dependent not only on their inherited genotype, but on mutations incurred within their parents' germline and their own development.

3.2 Selection Pressure

Evolutionary selection for or against alleles can alter both the genotype and allele frequencies.

3.2.1 Negative Selection

In the case of *negative selection,* if individuals with the disease (q^2) die before reproducing or are less able to reproduce, the allele frequency (q) will steadily decline.

3.2.2 Heterozygote Advantage

If there is a positive survival advantage for heterozygote carriers compared with normal individuals, referred to as a *"heterozygote advantage,"* then the allele frequency (q) may increase.

3.3 Genetic Drift

Within a small population, allele frequencies can change by random chance. For example, in a heterozygote cross the probability of having a homozygous child (aa) is 25%. However, if a heterozygote cross results in four children, three of whom are homozygous recessive (aa), then the allele frequency of a, f(a), increases. The effect of genetic drift is minimal in large populations, but can have a significant impact on allele frequency in small populations. If an affected person moves into a small, unaffected population, this, too, can dramatically change the allele frequency (the *"founder effect"*).

Connection to Microbiology

Individuals who are heterozygous for sickle cell trait are resistant to *Plasmodium falciparum* malaria. This gives them a selective advantage over normal homozygotes in areas where malaria is endemic. This explains how this deleterious allele (lethal in the homozygous condition) can persist in populations when natural selection against the homozygote would otherwise eradicate the trait.

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3.4 Gene Flow

In a given population, migration of one group of people into or out of that population may change allele frequencies, especially if a particular allele is more or less prevalent in the migrating population. Through time, gene flow within a population tends to make the people that make up that population more similar genetically to one another. As with genetic drift, the effect of gene flow is more pronounced in small populations.

3.5 Consanguinity

A consanguineous union occurs between mating individuals descended from a common ancestor. Such unions are more likely to produce offspring with recessive diseases because of the likelihood of shared disease-causing mutations. Statistically speaking:

- Siblings share 1/2 of their genes
- First cousins share 1/8 of their genes
- Second cousins share 1/32 of their genes

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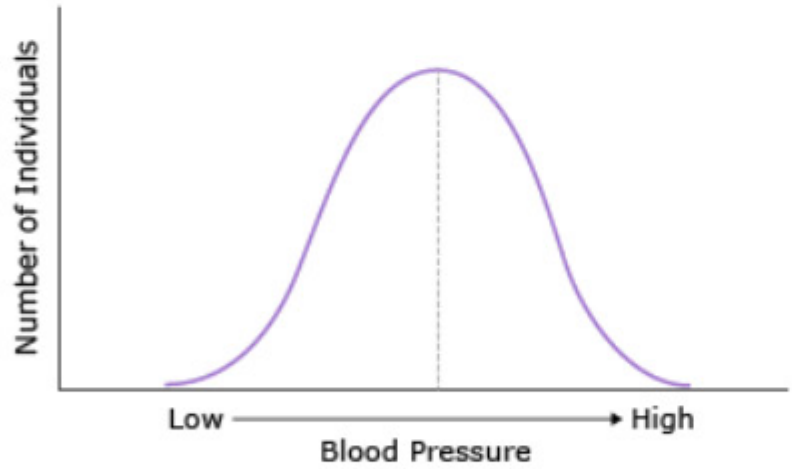
1 Overview of Multifactorial Diseases

Unlike the single-gene defects that we have discussed in previous chapters, most common human diseases are multifactorial: they have a genetic component, but they do not conform to Mendelian inheritance patterns because they have complex polygenic and environmental etiologies.

2 Multifactorial Inheritance

When there are multiple contributions (genetic and environmental) to the production of disease, the individual factors are referred to as *risk factors* and the sum of their contributions is that individual's *liability* for the disease. The distribution for these multifactorial diseases tends to follow a normal or bell-shaped curve. Blood pressure is an example of a multifactorial trait. There is a genetic component to the correlation between the blood pressures of parents and children; however, there are clearly environmental influences such as diet and stress which affect these findings.



▲ **Figure 5–2.0** Multifactorial Inheritance

2.1 Threshold Model

The multifactorial trait itself may not be a continuous spectrum. In some cases, the patient either has the disease or does not, such as lung cancer. The diagnosis of the disease is set by diagnostic criteria, and in these cases, disease occurs when a certain *liability threshold* has been achieved. In other words, for some individuals who have few of the alleles or environmental stimuli that would cause the disease, there is little chance of disease. Once some threshold for accumulated genetic and environmental factors is crossed, however, disease results.

USMLE® Key Concepts

For Step 1, you must be able to:

- Describe the risk factors and liabilities for most common multifactorial diseases.
- Explain the relationship between liability threshold and recurrence risk.
- Describe how multifactorial disease recurrence risk is altered by the severity of disease in affected family members, the gender of affected family members, and the prevalence of the disease in the population.
- Interpret the findings from twin and adoption studies concerning trait heritability.

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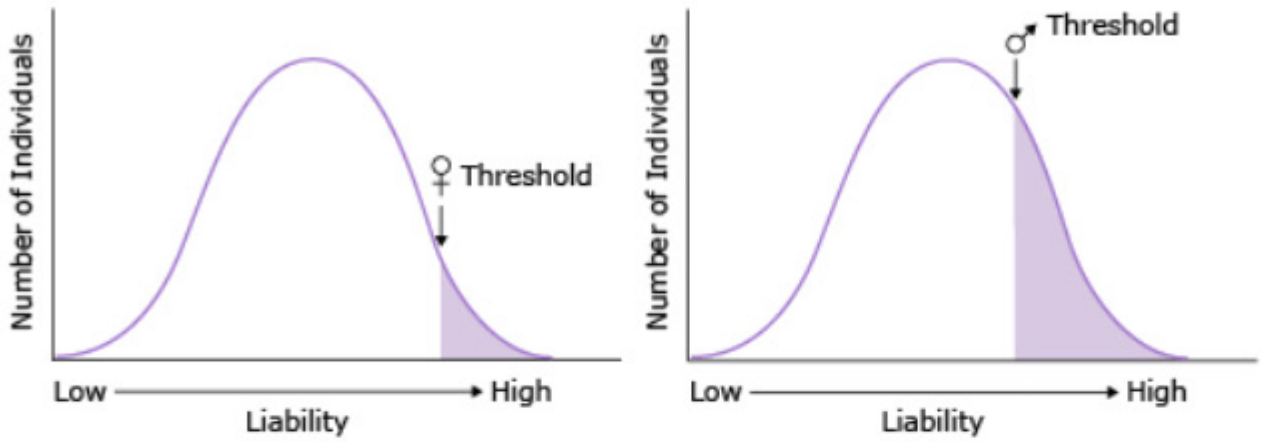
For some diseases, the thresholds for males and females are different. If the male threshold is lower than the female threshold, then the prevalence of the disease is higher in males than in females. The factors contributing to disease and the individual liability are usually determined empirically along with the recurrence risks. As an example, infantile pyloric stenosis has a higher liability threshold in females than in males. Therefore, the male always has the higher recurrence risk.



The male threshold is lower than the female threshold, so the prevalence of the disease is higher in males than in females.

▲ **Figure 5–2.1** Recurrence Risks for Infantile Pyloric Stenosis

2.2 Recurrence Risk for Multifactorial Diseases

The analysis of recurrence risks for single gene disorders is quite straightforward if one can construct a Punnett Square using the genotypes of the parents in a particular family, as we saw in chapter 1. In multifactorial diseases, the process is much more complex:

- Recurrence risks for multifactorial diseases must be determined empirically by direct observation of data in the population.
- Recurrence risk increases as the number of affected relatives increases. A family with a large number of affected members must be higher on the liability curve, with larger numbers of genetic and environmental risk factors.
- Recurrence risk increases as the severity of the disease expression in the proband increases, again indicating the family's relatively higher liability.
- Recurrence risk increases if the affected individual is a member of the more commonly affected gender.
- Recurrence risk increases as the prevalence of the disease increases in a population.
- Recurrence risk decreases rapidly for remotely related individuals.

2.3 Heritability

Heritability is defined as the proportion of the total variance of a trait that is caused by genes. This determination can be a major challenge in the complexity of the human genome and society, but two forms of studies are most frequently used: twin studies and adoption studies.

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2.3.1 Twin Studies

If a trait is purely genetically determined, we would anticipate that *monozygotic twins* (formed from a cleaved embryo and therefore genetically "identical" in the absence of mutational change) would share that trait 100% of the time: there would be *100% concordance*. In *dizygotic twins*, which are effectively siblings with 50% of their genes shared, there should be *50% concordance* (they would share the trait 50% of the time). For a trait which is purely determined by the environment, we would expect the same concordance in monozygotic and dizygotic twins as long as the pairs were raised together.

2.3.2 Adoption Studies

If a biological parent has a genetic trait but the child is adopted out to parents that are phenotypically normal, another measurement can be made of the relative roles of genetics versus the environment. For example, if the children of a schizophrenic biological parent are raised by non-schizophrenic adoptive parents, 8–10% will develop schizophrenia. If the biological offspring of non-schizophrenic parents are raised by non-schizophrenic adoptive parents, only 1% of those children will develop the disease. This provides evidence that there is at least some genetic component to that disease.

2.4 Familial vs. Sporadic Incidence

Familial cases of genetic disease involve changes in germ-line DNA, which are inherited through generations. Sporadic somatic mutations in tumor suppressor genes and protooncogenes plays a key role in common non-inherited cancers such as breast and colon cancer, and mutations in these same genes in the germline cause inheritable cancers.

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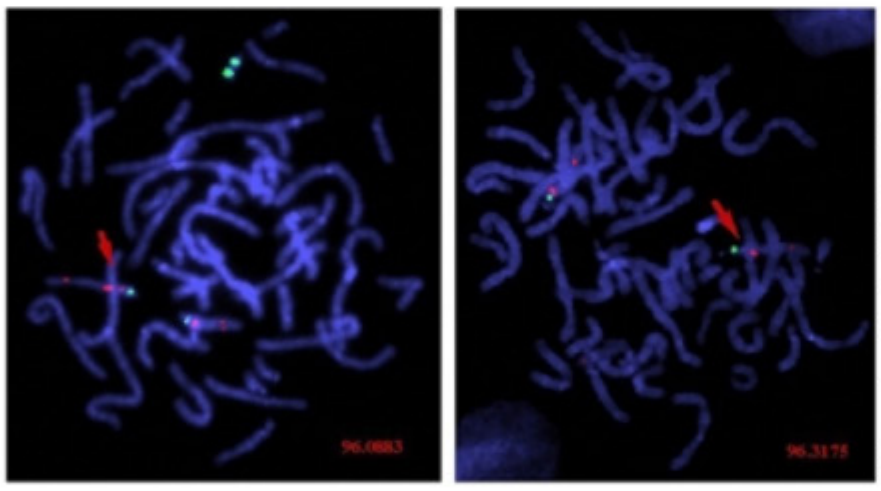
1 Overview of Gene Mapping

Gene mapping refers to the localization of genes to specific sites on chromosomes. It is a critical step in the understanding, diagnosis, and eventual treatment of genetic diseases. Once the location of a disease-causing gene is determined, more detailed investigations can be carried out, up to and including gene cloning and gene therapy.

1.1 Physical Mapping

Physical mapping is any technique that determines the absolute position of a gene on a chromosome, not just its position relative to known markers. One often-utilized technique in physical mapping is *fluorescence in-situ hybridization (FISH)*, in which chromosome-specific DNA segments are tagged with fluorescent dyes, hybridized with the patient's chromosomes, and then observed under a fluorescence microscope. Since the probe will only bind with a complementary DNA sequence, specific chromosomes can be identified and counted, using regular karyotyping techniques, and deletions or duplications of the probed region can be detected.



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▲ **Figure 6–1.1** Fluorescence In Situ Hybridization

1.2 Linkage Analysis

When homologous chromosomes exchange portions of their DNA during prophase I, a process termed *crossover*, new combinations of alleles are formed. Crossovers are more likely to occur between loci on the chromosome that are far apart than those that are close together or "linked." With the increased knowledge of the human genome, thousands of allelic polymorphisms are known. Polymorphisms are benign differences in each person's genetic code and can be used as markers if they are known to be close to a disease allele. If the disease gene cannot be assayed directly, then the marker polymorphism can be used to predict the inheritance of the disease gene.

USMLE® Key Concepts

For Step 1, you must be able to:

- Explain the diagnostic uses of, and interpret data from, fluorescence in-situ hybridization.
- Analyze genetic linkage using polymorphic markers such as RFLPs.

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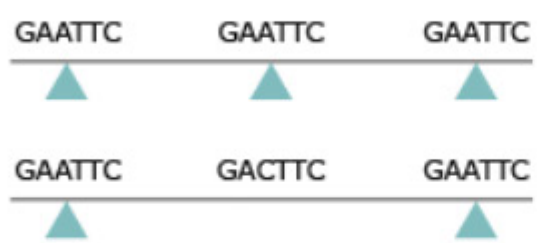
1.2.1 Polymorphic Genetic Markers

Various types of polymorphic markers are currently available for the purpose of recombination mapping:

Restriction Fragment Length Polymorphisms (RFLPs) Restriction endonucleases are enzymes that cut DNA at specific palindromic sequences. When DNA from different individuals is subjected to digestion by these enzymes, benign variations in their nucleotide sequences will lead to different fragment lengths, generating unique patterns for each person depending on the specific markers present.

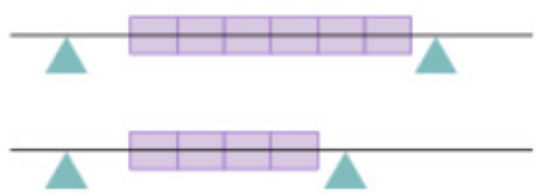
In an RFLP, the presence or absence of a restriction site (▲) produces DNA fragments of varying lengths, reflecting sequence variation.



▲ **Figure 6–1.2A** Restriction Fragment Length Polymorphisms

Variable Number of Tandem Repeats (VNTRs) Less often used, this technique also makes use of differences in nucleotide sequences among individuals. In this case, varying numbers of repeating units from 20–70 bases, each in a specific region of a chromosome, are used as markers.

In a VNTR, variation in fragment lengths is produced by differences in the number of tandem repeats located between two restriction sites (▲).



▲ **Figure 6–1.2B** Variable Number of Tandem Repeats

Short Tandem Repeat Polymorphisms (STRPs, microsatellites) Similar to VNTRs, these 2–6 base pair long segments are amplified by polymerase chain reaction (PCR), then digested by restriction endonucleases to generate a unique pattern of fragments, similar to RFLP analysis. This technique is currently used in paternity testing and some forensic analysis.

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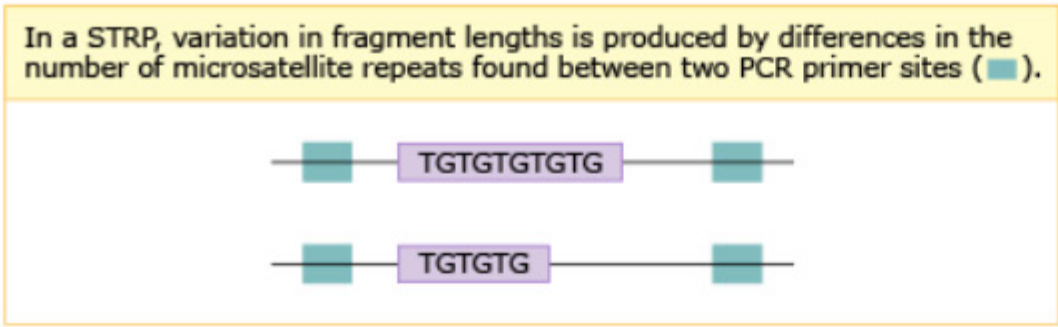
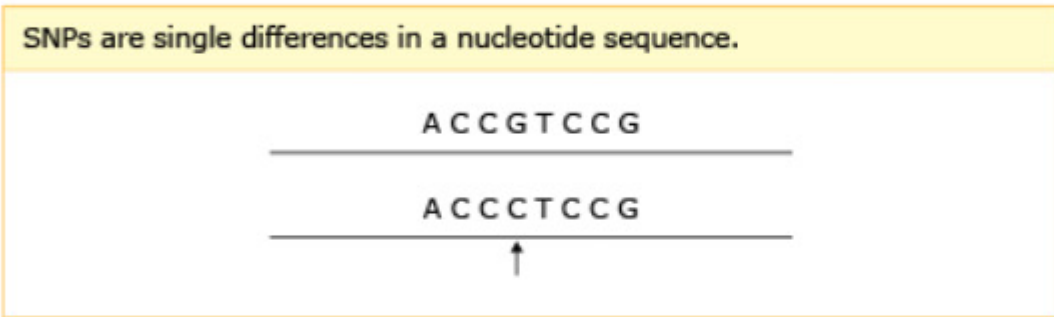


▲ **Figure 6–1.2C** Short Tandem Repeat Polymorphisms

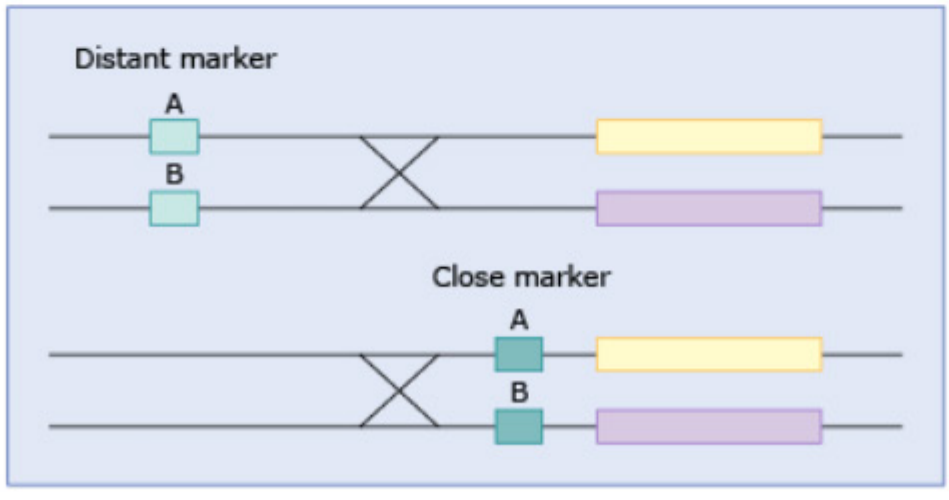
Single Nucleotide Polymorphisms (SNPs) The most specific markers of all, SNPs are found in a specific position in the DNA where more than one nucleotide is found in the normal population (e.g., A or T). Countless SNPs have been characterized, allowing for precise gene mapping. These must be identified by DNA sequencing.



▲ **Figure 6–1.2D** Single Nucleotide Polymorphisms

1.2.2 Linkage

The term genetic linkage refers to the probability that two traits will be inherited together. One might expect that two genes on the same chromosome would always be linked—that is, inherited together 100% of the time. However, during meiosis, alleles undergo rearrangement due to the process of recombination. The further apart two loci or polymorphic markers are from each other, the greater the chance they will be affected by recombination events and the less likely they are to be inherited together.



▲ **Figure 6–1.2E** Linkage

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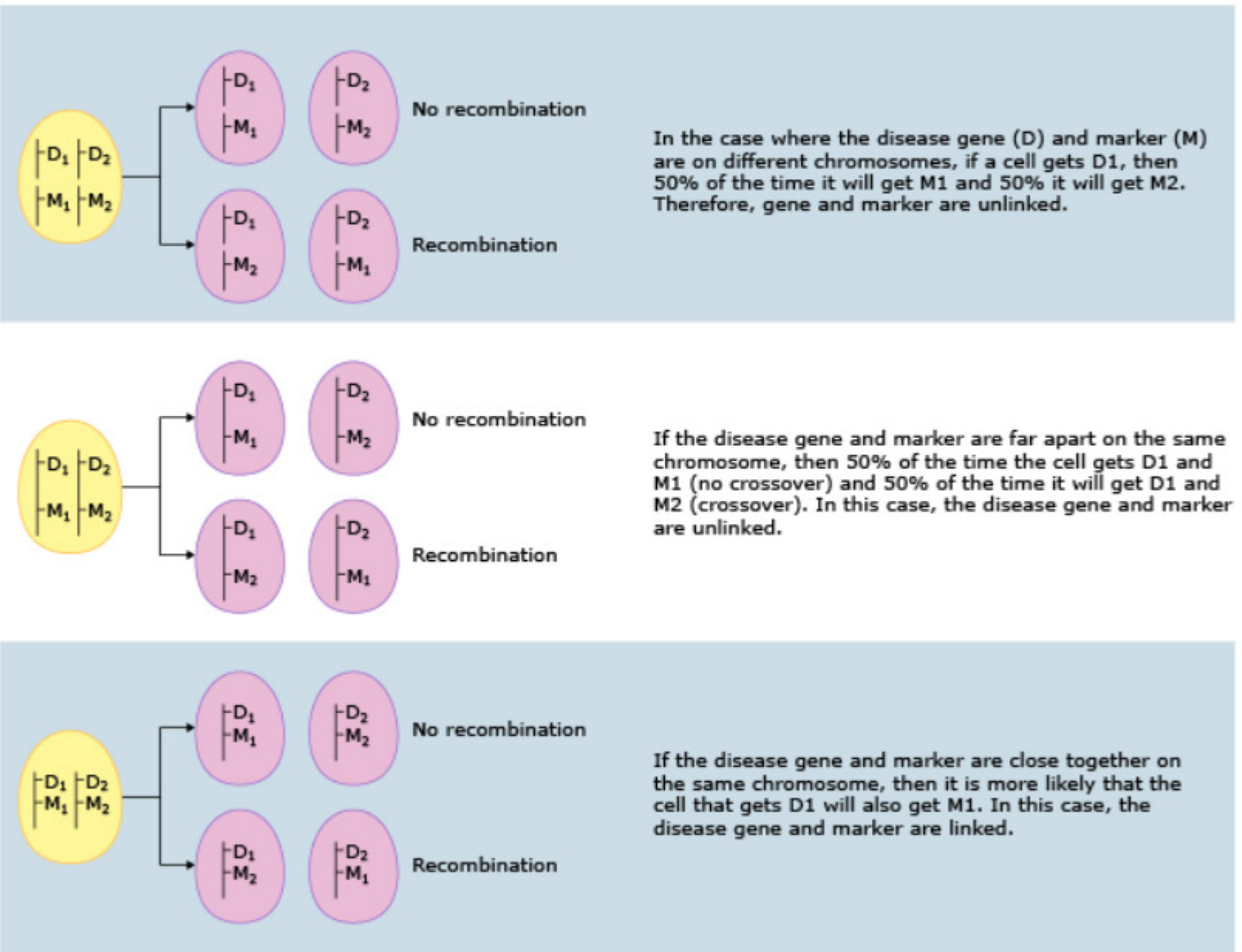
Based on this knowledge, one can calculate relative distances between two genetic loci or markers by studying the frequency of recombination between them. Specifically, a 1% frequency of recombination is defined as a distance of one centimorgan (cM). This is not directly proportional to the actual distance along the chromosome, as some areas undergo recombination at higher rates.

In addition, these principles can be used to perform a *linkage analysis*. A linkage analysis utilizes recombination frequencies between disease causing genes and various markers of known chromosomal locations. If a particular genetic marker is found to be in strong genetic linkage (has a very low recombination frequency) with disease, one can assume the disease-causing gene is located physically in close proximity to that marker.



▲ **Figure 6–1.2F** Linkage Analysis

1.2.3 Log of the Odds (LOD) Scores

Recombination frequencies can be extremely difficult to measure in small populations. Therefore, geneticists often make use of large populations and a statistical measure called the *log of the odds (LOD)* score to estimate genetic linkage. The LOD score is calculated by computer and is beyond the scope of Step 1, however a LOD score greater than 3 indicates that linkage is present, whereas scores less than 2 suggest no genetic linkage.

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Genetic Diagnosis and Therapy

1 Overview of Genetic Diagnostics

Genetic diagnostics use molecular techniques to directly identify the disease causing mutation in the individual who is being tested. These tests are highly accurate provided that the molecular nature of the disease in the population has been identified. These tests have application in carrier diagnosis in recessive diseases, prenatal diagnosis, preimplantation testing, and in cases in which it is necessary to detect hidden alleles affected by reduced penetrance or late onset of disease.

2 Prenatal Genetic Diagnosis

Armed with the knowledge of how genetic mutations can lead to disease, it is possible to predict which individuals may be affected by certain disorders. For this reason, many pregnant patients may want such genetic information on their developing fetuses.

2.1 Preimplantation Diagnosis

Following in vitro fertilization, embryos at the eight-cell stage can be tested for known genetic defects. One cell from the embryo is removed, the DNA is extracted and amplified by PCR, and gene testing is performed. Based on the findings, only embryos without the mutation are implanted.

2.2 Chorionic Villus Sampling

Chorionic villus sampling is a technique that involves biopsy of the developing trophoblast to obtain genetic material for analysis. It can be performed very early in pregnancy, around the ninth week of gestation. However, there is a risk of obtaining maternal tissue and getting false positive results, as well as a risk of fetal demise (1.9/100).

2.3 Amniocentesis

Amniocentesis is an in utero test which involves sampling of the amniotic fluid for fetal cells that can be used for genetic analysis. There is little risk of obtaining maternal tissue, but the test cannot be performed until about the 14th week of gestation. It produces a low risk for loss of the fetus (1.4/100).

USMLE® Key Concepts

For Step 1, you must be able to:

- Explain the principles of diagnosis for human genetic disorders.
- Interpret the results of commonly used genetic tests to predict genotype prenatally.
- Describe potential genetic strategies for treating disease.

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3 Diagnosis of Cytogenetic Abnormalities

3.1 Fluorescence in Situ Hybridization

Fluorescence in situ hybridization (FISH) was discussed as a means of physical genetic mapping in chapter 6. Likewise, it can be used with specific DNA probes as a means of genetic diagnosis to identify specific deletions and cytogenetic changes. For example, in DiGeorge or 22q deletion syndrome, a specific probe can identify loss of one of the copies of the 22q region.

3.2 Spectral Karyotyping

In spectral karyotyping, different fluorescent probes are used that bind differently to specific sets of chromosomes. This process produces a karyotype in which each chromosome is stained a different color, allowing the easy visualization of translocations and rearrangements.

▲ **Figure 7–2.2** Spectral Karyotyping

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4 Direct Genetic Diagnosis

4.1 Gel Electrophoresis of PCR Products

In cases in which a mutation alters the size of a gene, such differences can be detected using PCR-amplified DNA subjected to gel electrophoresis. For example, in diseases that result from trinucleotide repeat expansions, the larger size of the defective gene can be observed by its migration pattern in the gel. Large products migrate more slowly and will therefore be higher up on the gel.

4.2 PCR and Allele-Specific Oligonucleotide (ASO) Probes

Allele-specific oligonucleotides (ASOs) are short nucleotide sequences that have been created to bind specifically to known disease alleles. If ASOs for the disease allele and normal allele are used to probe PCR-amplified patient DNA on a dot blot, the genotype of the individual can be determined.

4.3 DNA Chips

The computerization and miniaturization of ASO probe technology involves embedding thousands of different probes on a silicone chip. When a patient's DNA is amplified with PCR and tagged with fluorescent labels, the sites of binding to the chip can then be analyzed and recorded by computer.

4.4 Restriction Fragment Length Polymorphism Analysis of PCR Products

If a mutation either destroys or creates an endonuclease site, this can alter the size of DNA fragments, which would result after treatment with a restriction enzyme. These different sized fragments, referred to as *restriction fragment length polymorphisms (RFLP)*, can be detected by gel electrophoresis. These can be particularly useful in the analysis of trinucleotide repeat expansion diseases, since the expanded sequence can be too large to be conveniently amplified by PCR.

4.5 Direct DNA Sequencing

Sequencing for known disease genes has become more rapid and inexpensive, and when a single gene disease is suspected it is now often the first line test. In addition, multiple genes that cause a similar phenotype are grouped into disease gene panels—for example, all the genes that cause infantile-onset seizures are available as one test. The limitations for this testing include that the clinician needs to know either the specific gene to have sequenced, or to have a panel available for the phenotype. Sequencing can also be complicated by genetic changes of uncertain significance; it is not always clear if these differences could be benign polymorphisms or novel disease-causing mutations.

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4.6 Whole Exome and Whole Genome Sequencing

It is now possible through next generation sequencing to examine the genetic sequence of every exome (coding region) in every gene or even the entire genome. This is most appropriate either for cases where all other directed testing has been negative, or to find novel genes that have not yet been described as associated with a disease. Since the volume of information that is generated is on a much larger scale that traditional sequencing there are often many genetic changes of uncertain significance that require further research to determine if they are clinically relevant. In addition it is possible to have unexpected incidental findings, such as cancer predisposition or early-onset dementia genes, which may have significant clinical implications, but are unrelated to the reason for testing.

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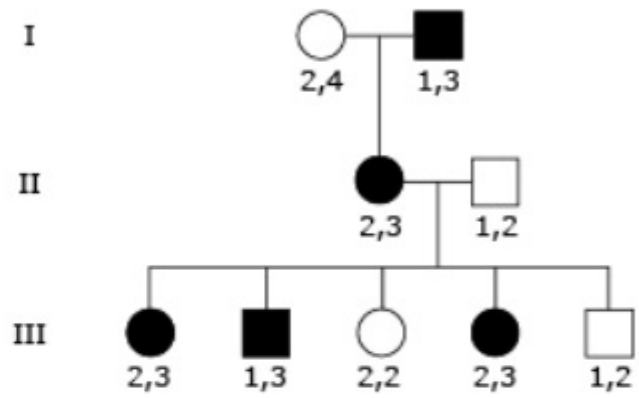
5 Indirect Genetic Diagnosis

Indirect genetic diagnosis can be used to infer patient genotypes based on analysis of the linkage phase of specific markers to the disease allele. This process requires pedigree information and may be affected by recombination, but it benefits from the fact that multiple mutations can be assayed with a single test, and the disease mutation itself does not need to be known. The same polymorphisms used for gene mapping, described in chapter 6, can be used here for the identification of linkage between the STRP or RFLP and the disease allele.

5.1 Using STRP

In a family transmitting a genetic disease, each member has been typed for a short tandem repeat polymorphism that is closely linked with the disease gene. The genotypes for these STRP are shown below each symbol.

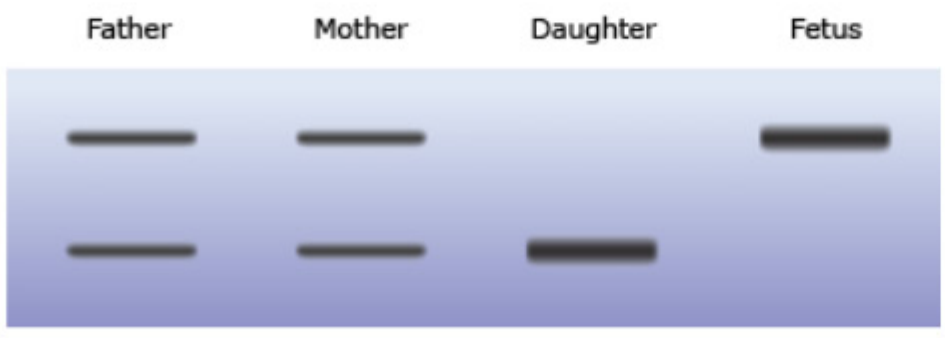
If one follows the linkage of the STRP carefully through the pedigree, it must be true that a "3" from the father is linked with the disease allele. This should allow for the prediction of a family's recurrence risk, which would be much closer to 100% or 0% than the standard 50% for an autosomal dominant disorder. It is important to remember that crossover can happen even between tightly-linked regions so this is subject to false-positive and false-negative results.



▲ **Figure 7–5.1** Indirect Genetic Diagnosis Using STRP

5.2 Using RFLPs

Restriction fragment length polymorphisms can be used for direct diagnosis if the mutation causes addition or deletion of endonuclease sites in the DNA, but RFLP also can be used as an indirect genetic test. In the indirect usage, the endonuclease site is closely linked to the disease allele, but is not actually the same mutation that is causing the disease. Therefore, it is possible, using the indirect test, that recombination could occur that would lead to an incorrect conclusion. If an RFLP is used as a marker for a disease gene, the results can be analyzed using Southern blot and a gene probe. In the case illustrated in figure 7–5.2, father and mother are heterozygotes for an autosomal recessive condition. The daughter is homozygous normal. Using RFLP and Southern blot in this setting, the fetus can be seen to be homozygous recessive and should be affected with the disease.



▲ **Figure 7–5.2** Indirect Genetic Diagnosis Using RFLPs

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6 Gene Therapy

Considering the number of incurable genetic disorders that exist, genetic therapies hold incredible potential for transforming medicine. In gene replacement therapy, the underlying idea is to add-back a normal gene in loss-of-function disorders caused by a lack of a particular protein. In order to accomplish this, a DNA vector (usually derived from a virus) is engineered to contain the gene of interest. The vector is then delivered to a patient with the hope that the engineered gene will be integrated into the genomic DNA.

Currently, gene replacement therapy has been used successfully to treat one form of severe combined immunodeficiency (SCID) caused by the lack of a functional adenosine deaminase (ADA) gene. Although the technique at present can only "add-back" normal genes in loss-of-function disorders, true gene replacement may be able to be achieved to treat genetic disorders arising from other mechanisms. Additionally, because this therapy involves insertion of DNA into the genome, there exists a small but significant possibility that the integration event may disrupt genes and promote cancer. For example, leukemia developed in four of the ten patients in the original SCID trial with ADA-containing viral vectors.

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